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Evaluation of Anticancer activity of hydroalcoholic extract of *Cassia occidentalis* (CO-A002) in 3 human cancer cell lines

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Abstract

To evaluate the *in vitro* anticancer activity of hydroalcoholic extracts of *Cassia occidentalis* against 3 human cancer cell lines from different tissues.

Introduction

Cancer is an alarming disease and quite lethal in nature in developed and developing countries. A lot of new therapeutic components and therapies are available in the market but have some side effects on human beings' organs. Cancer therapy has been characterized throughout history by ups and downs, not only due to the ineffectiveness of treatments and side effects but also by the reality of complete remission and cure in no. of cases. Radiation and anti-cancer medications, which have been the preferred course of treatment, are part of the therapeutic arsenal together with surgery in the case of solid tumors. Immunotherapy has emerged as a significant therapeutic alternative in recent years and is currently the treatment of choice in many situations. Herbs have been used since the beginning of human civilization for medical treatment and form the basis of modern medicine.

In vitro anticancer potential of hydroalcohlic leave extract of *Cassia occidentalis* at concentration of 100 µg/ml was evaluated against eight human cancer cell lines- MCF-7 ,HCT-116, , PC-3, from three different origins (breast, colon, and prostate gland) using MTT assay

Material and Methods:

In vitro cytotoxicity against the human cancer cells, cultured for 48h in presence of different concentrations of *C. occidentalis* extracts and percentage of cell viability, was evaluated using MTT assay.

Results:

It was observed that aqueous extract of *C. occidentalis* had more potential than hydro-alcoholic and alcoholic extracts against HCT-15, SW-620, PC-3, MCF-7, SiHa, and OVCAR-5 human cancer cell lines at 100, 30, and 10 µg/ml in a dose-dependent manner.

Keywords: The plant can be explored for the possible development of lead molecules for drug discovery.
Key-Words: cell lines, CO-A002, In-vitro, cancer

Introduction

Cancer is a disease of metazoan referred to uncontrolled multiplication of normal human cells. It is one of the leading causes of death around the globe. It is increasing in economically developing countries as a result of aging, smoking, physical inactivity, metabolic, Chemical, environmental, genetic factors, and “westernized” diets. The adage "Prevention is always better than cure" is especially relevant when it comes to cancer because, when possible, a cure for the disease is linked to high cytotoxic burdens and/or intrusive treatments (Bertram, 2000; Amin and Mousa, 2007).

For the past decades, much research has been conducted in order to discover natural compounds with potential anticancer activity and several plant-derived agents (e.g., paclitaxel, docetaxel; vinblastine, vincristine; topotecan, irinotecan, etoposide, etc.) have been successfully used for treatments of cancer. Among the anticancer medications, 69% of drugs approved between 1940 and 2002 are either natural products or developed that based on knowledge obtained from natural products.

The application of plants for the treatment of cancer s appears to be inevitable, building the basis for modern medical science and imparting a great source for new drugs. Approximately 60% of drugs presently used for the treatment of cancer have been isolated from herbs (Gordaliza, 2007).

Flavonoids are naturally occurring polyphenolic metabolites found throughout the plant kingdom. Flavonoids are regarded as safe and easily obtainable, making them ideal candidates for cancer chemoprevention or associated agents in clinical treatment as herbal products. Almost all artificial agents currently being used in cancer therapy are highly toxic and produce more damage to normal cells. The ideal anticancer agent would exert minimal adverse effects on normal tissues with the maximal capacity to inhibit tumor growth or to kill tumor cells.

Flavonoids have been found to possess both anti- and prooxidant action because of their polyphenolic structure. While antioxidant effect and capability to remove reactive oxygen species (ROS) have been shown to account for most of the reported biological effects of phenolic compounds. Recent research has shown that the anticancer properties of flavonoids may be achieved via prooxidant activity. Compared to normal cells, cancer cells demonstrate a higher and more permanent level of oxidative stress, making malignant cells more susceptible to being killed by medications that cause elevated ROS levels.

Radiation therapy and surgery areused to treat localized cancers whereas chemotherapy is used to treat metastasis type of cancer because cells travel in the body through the bloodstream. The drugs commonly used for the treatment of cancer such as antimetabolites, antibiotics, and even hormones. Acute lymphoblastic and acute myelogenous leukemia, Hodgkin's germ cell cancer, Hodgkin's and non- Hodgkin's lymphoma, small cell lung cancer, choriocarcinoma, and ovarian cancer are some advanced cancers that can be cured with chemotherapy. Chemotherapeutic agents are cytotoxic and, apart from affecting tumor cells, these active principles also have an impact on rapidly dividing normal cells, and exhibit side effects in theform of myelosuppression, nausea, alopecia, and vomiting.

According to Rao *et al* (2005), the efficacy of cancer drugs is often limited by their insolubility and the low rate at which the tissue absorbs them, and the drug resistance of tumor. Antitumor drugs have also been associated with the development of secondary malignant. Plants have long been used to cure cancer, and scientists are still looking to nature for new chemotherapeutic drugs. The advancement of science and technology in the search for novel anticancer drugs has boosted current research and development geared at finding new antiproliferative agents from natural sources.

Morita *et al* (2005), elucidated that antimutagenic factors are present in no. of vegetables and fruits. In this study, Olive leaf aqueous extracts were screened for their antimutagenic activity against sodium azide and nitrofluorene by the Ames test in the presence and absence of S9. Numerous studies have demonstrated that various fruits and vegetables carry phytochemicals that have bioactive properties. Therefore, there are naturally occurring bioactive factors in several vegetables and fruits.

According to estimates, the total number of new cases of cancer will increase from 10 million in the year 2000 by roughly 25% in each decade to reach 24 million in the year 2050; the total number of deaths will increase from 6 million in the year 2000 to 10 million in the year 2020 to over 16 million in the year 2050; and there will be 17 million new cases of cancer in less developed nations in the year 2050, compared to only 7 million new cases in developed nations (Akindele *et al*, 2015).

Cassia occidentalis (Family: Caesalpiniaceae) plant has been extensively used in indigenous and folk-lore medicine systems. The leaves and seeds of Cassia occidentalis are found to be used in constipation, ringworm, and cough in the Ayurvedic system of medicine. It is a rich source of flavonoids, phenols, and anthraquinone glycosides. Hence the present study was designed to evaluate the antioxidant and cytotoxic effect of hydroalcoholic extract of *Cassia occidentalis* leaves. *Cassia occidentalis* (COA002) is an annual plant widely distributed in Africa, America and India along the roadside barren areas. The plant is commonly called “kasmada, Kasondi, Bardihaedma. The plant is used as hepatoprotective (Sharma *et al*, 2000), anti-inflammatory, CNS depressant, and analgesic, in vitro anticarcinogenic (Tona *et al*, 2004) and anti-helminthic and antiplasmodial (Tona *et al*,1999) activities of extracts of the plant had been reported.Reports for its immunosuppressive, antimutagenic, anti-inflammatory, anti-dermatophyte, antibacterial, antiplasmodial, anti-fertility, antimalarial, and antidiabetic activities had been published. It has been claimed that the plant's leaf extracts can restore, safeguard, and normalize liver functions. Considering the therapeutic values of *C. occidentalis*, the present work was undertaken to evaluate the *in vitro* cytotoxic and antibacterial activity.

Materials and Methods

Preparation of plant extract:

Cassia occidentalis leaves are collected from near the bank of Chenab River Akhnoor, Jammu. The plant material was identified and authenticated in the Department of Botany IIIM, Jammu. The leaves were shade dried and then pulverized into a fine powder using a milling machine (Retsch GmbH, Germany). 100 gm of leaf powder was extracted with 500 ml of 50% ethanol (v/v). The extract was filtered using Whatman filter paper and solvent was evaporated using a rotary evaporator (RE121 Buchi, Switzerland). The resultant extract power was dried using a freeze-dryer (Labconco, USA).

Chemicals for anticancer

These were RPMI-1640, minimum essential medium (MEM), fetal calf serum, trypsin, trypan blue, ethanol, penicillin, streptomycin, gentamycin, dimethyl sulfoxide (DMSO), sulforhodamine, mitomycin-C, paclitaxel, and 5-fluorouracil (SIGMA Chemical Co., USA); phosphate buffer saline (PBS, MERCK, Germany); trichloroacetic acid (TCA), distilled water, sodium hydroxide, Tris-EDTA buffer, Tris buffer (Hi-Media); acetic acid, sodium bicarbonate, hydrochloric acid (RANKEM, New Delhi, India), isopropanol (SISCO, Mumbai, India), and Tris-acetate-EDTA buffer. All additional chemicals utilized in this study were locally purchased and of analytical quality.

Procedure:

Cell culture, growth conditions and treatments

Human breast cancer MCF-7, colon cancer, HCT-116 and prostate cancer PC-3 cells were purchased from Sigma Aldrich, India (European

Collection of Cell Cultures, ECACC). Cells were grown in RPMI/MEM growth medium containing 10% heat inactivated fetal bovine serum (FBS), penicillin (100 units/ml), streptomycin (100 µg/ml), L-glutamine (0.3 mg/ml) and $NaHCO_3$(3.8mg/ml). Cells were grown in CO_2 incubator (Thermocon Electron Corporation, Houston, TX) at 37^0C with 95% humidity and 5% CO_2 gas environment. Adherent cells grown in monolayer cultures were detached with trypsin (0.1% w/v) / EDTA (1mM) solution. Soon after cells were ready to detach, the trypsin / EDTA solution was removed. Cells were dispersed gently by pipetting in complete growth medium, centrifuged at 200xg, 4^oC for 5min. A 96-well plate was filled with the necessary cell suspension (0.6x104/100 l), which was then cultured in a CO2 incubator. After 16 hours, fresh complete media was added to the cells. The tested substance was administered to cell cultures that were at the semi-confluent stage (about 70% confluent), while the untreated control cultures merely got DMSO (less than 1%).

The *in vitro* cytotoxicity of the extracts of CO-A002 was determined by semiautomated assay using sulforhodamine-B (SRB). The human cancer cell lines were grown in tissue culture flasks at 37°C in an atmosphere of 5% CO2 and 90% relative humidity in complete growth medium. Cells from selected flasks with subconfluent growth stages were collected using trypsin-EDTA. Using a hemocytometer, the number of cells per milliliter of suspension was determined. The cell density of 10,000 cells/100 L, or the appropriate number for each cell line, was adjusted in the cell suspension. Using handy-step, 100 L of cell suspension was added to each well of 96-well plates. The plates were incubated for 24 hours at 37°C in an environment with 5% CO2 and 90% relative humidity. Then, 100 µL of the working solution of each test substance was added to the 96-well plates' wells. The stock solutions of the extracts (20 mg/mL) were prepared in DMSO and serially diluted with complete growth medium such that 100 µL of working solutions of each extract gave concentrations of 10, 30, and 100 µg/mL (final DMSO concentration was 0.5% highest to 0.001% lowest) added to the 96-well cell culture plates. The 96-well cell culture plates contained appropriately seeded cells (e.g., 8000 cells/100 µL for HCT-116 and A431; 10000 cells/100 µL for HeLa) and all vehicle controls contained the same concentration of DMSO.

The plates were incubated for 48 h at 37°C in an atmosphere of 5% CO2 and 90% relative humidity. Thereafter, 50 µL of chilled 50% TCA was gently added to each well of the plates, making a final concentration of 10%. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. The plates were then washed 5-6 times with distilled water and thereafter air-dried. To each well, 100 µL of SRB dye (0.4% wt/vol in 1% acetic acid) was added and left at room temperature for 30 min. Thereafter, the plates were washed with 1% acetic acid. The plates were again air-dried and 100 µL of Tris buffer (10 mM; pH 10.5) was added to each well. The plates were shaken gently for 10–15 min. on a mechanical shaker. Blank wells contained medium, but no cells and the control wells contained cells but no test samples. The optical density (OD) of the plate wells was recorded with a microplate reader at 540 nm and data were maintained. Growth inhibition was calculated as the percent survival of treated cells over control cells × 100 (T/C):

% Growth inhibition = 100 - [OD(test sample) – OD(blank)]/OD(Control)- OD (blank) x100

Statistical Analysis

The results obtained in this study are displayed as mean ± SEM. Data analysis was done using one-way ANOVA followed by Dunnett's multiple comparison test (GraphPad Prism 5, GraphPad Software Inc., La Jolla, CA, USA). Values were considered significant at $P<0.05$

Results and Discussion

Medicinal plants have been traditionally applied in folk medicine for centuries as natural healing therapies with remarkable proven therapeutic effects in many areas like the prevention of

cardiovascular diseases (Mashour *et al*, 1998) and anti-inflammatory, antimicrobial, and anticancer activity.

Although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming increasingly recognized that the beneficial effects of plants are due to a complex interplay of the composite mixture of compounds present in the whole plant (additive/synergistic and/or antagonistic) rather than constituent single agents alone (Liu, 2003; Karna *et al*, 2012).

Cancer therapy either radiotherapy or surgery is quite effective if the cancer is detected at early stage but many cancers are still diagnosed if cells from a primary tumor have already metastasized to other parts of the body and then chemotherapy is used. Chemotherapy refers to delivering drugs systemically and hence drugs can reach and kill the cancer cells, but most of these drugs cause severe side effects in patients and so drugs should be used at low levels (Senapati *et al*, 2018).

According to Jemal *et al* (2010), the low efficacy of chemotherapy in patients with advanced cancers is reflected in the low 5-year survival rates observed in these patients.

Denny and Wansbrough (2010) said that the main challenge is to design new drugs which will be more selective and effective for cancer cells and hence have low side effects

More than half of cancer patients utilise some form of integrative therapy according to meta-analysis of integrative cancer treatments. Patients may resort to integrative therapies if their condition does not respond to conventional medical treatment (Lammersfeld, 2014).

According to Rates (2001) and Jemal *et al* (2010) the alternative therapies using herbal products is increasing, particularly those derived from herbs, because of the high number of cancer cases globally. Many plant extracts and active molecules have been studied *in vitro* cancer models to look for new sources of therapeutic anticancer agents.

Cell proliferation Assay

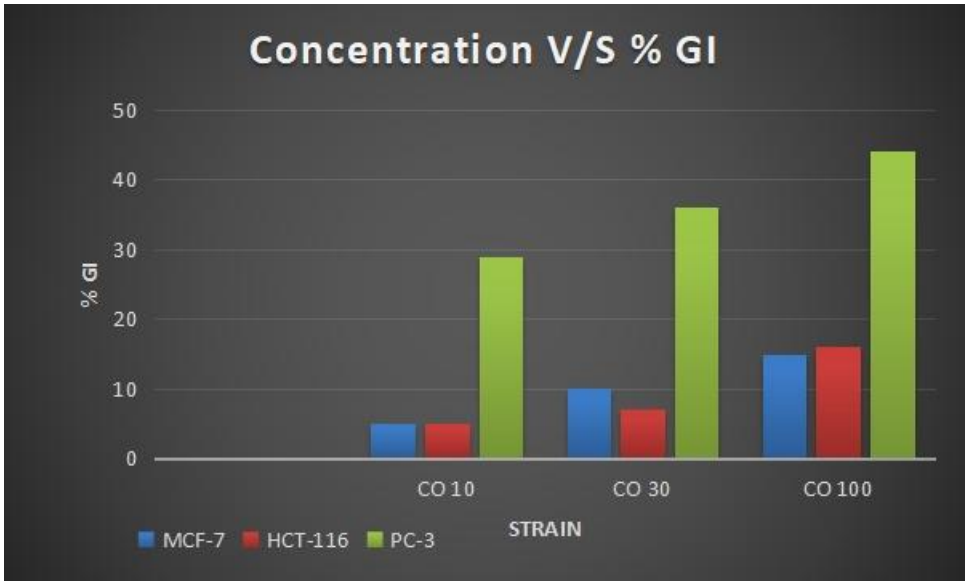
A quantitative colorimetric approach for assessing cell survival and proliferation is the MTT test. The metabolism of living cells is the evaluated parameter. After being dissolved in DMSO, the dark blue, water-insoluble formazan that is produced by metabolically active cells from the light-yellow tetrazolium salt (MTT) may be directly measured (Vistica*etal*,1991; Maehara*etal*,1987). The number of live cells is directly correlated with the formazan's absorbance. Overnight, cells were plated in 96-well plates at a density of 6000 cells per well in 100 µL of the medium. The day after, cells were exposed to the test compound at concentrations of 10, 30, and 100 M for 48 hours. For 4 hours, the dye [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) was added at a concentration of 2.5 mg/ml. MTT-formazon crystals were dissolved in 150 µL of DMSO after the supernatant was aspirated, and OD was measured on an ELISA reader (BioTek.) at 540 nm (reference wavelength, 620 nm). Cell growth was assessed by comparing the absorbance of treated and untreated cells.

Cell lines were treated with test compounds at 10, 30 and 100 µM concentrations for 48 h. The tested compound was found to be non-cytotoxic in breast cancer MCF-7 and colon cancer HCT-116,whereas it was found to be active at human prostate cancer PC-3 cells with 36% and 44% cells growth inhibition at 10 and 30 µM, respectively.

No report of anticancer activity of *Cassia occidentalis* (COA002) has seen from extensive literature search. However, other species have been investigated and reported.

Table: 1 COA002 Show Growth inhibition %age in MCF-7, HCT-116 and PC-3 Cell lines.

Conc. In µg/ml	**MCF-7** %GI	**HCT-116**%GI	**PC-3 %GI**
COA002- 10	5	5	**29**
COA002- 30	10	7	**36**
COA002- 100	15	16	**44**



Integrative medicine with the approach of combining conventional western medicine along with alternative treatments like yoga, acupuncture, massages, herbal medicine, and stress reduction techniques, is being used to complement clinical medicines and treatment approaches in the management of cancer patients.Patients can turn to integrative therapies if the disease they are battling do not respond to traditional medical therapies and/or to help reduce symptoms while improving overall well-being (Lazar and Connor (1997).

Conclusion

The application of dietary and herbal phytochemicals as potential therapies against different cancers demonstrated antiproliferative activity through a variety of mechanisms of action, such as downregulation of PEG-2, activation of MAPKs and JNKs, COX-2 inhibition, stimulation of oncogenes, cell cycle arrest, reduced mitochondrial membrane potential, rise of ROS, etc. These dietary and herbal compounds, which were obtained from a variety of sources, demonstrated their anticancer properties both singly and in combination. They also markedly improved the chemopreventive ability against cancer cells.

Based on the results of MTT *in vitro* cytotoxicity assay in Human breast cancer (MCF-7), colon cancer (HCT-116) and prostate cancer (PC-3) cells, it can be concluded that the hydroalcohlic leave extract of Cassia occidentalis, (CO-A002) was found to be non-cytotoxic in cancer like breast cancer(MCF-7) and colon cancer(HCT-116) whereas it was found to be active at human prostate cancer (PC-3) cells with 36% and 44% cells growth inhibition at 30 and 100 µg conc. respectively.

Thus, further research should concentrate moreon the identification, isolation, and characterization of the specific bioactive molecules accountable for antitumor activity and their precise mechanism(s) of action or on the combined investigation of phytochemicals for a better result against cancers.

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