# Evaluation of Anticancer Properties of Medicinal Plants from the Indian Sub-Continent

**Article** *in* Molecular and Cellular Pharmacology · January 2011

DOI: 10.4255/mcpharmacol.11.04 · Source: DOAJ



**4 authors**, including:

Yunus Mohammad
ALANUS Hochschule und Bildungswerk gemeinnuetzige GmbH
**1** PUBLICATION **67** CITATIONS
SEE PROFILE

Abbas Ali Mahdi
King George's Medical University
**493** PUBLICATIONS **13,978** CITATIONS
SEE PROFILE

Sanjay Gupta
Case Western Reserve University
**401** PUBLICATIONS **15,516** CITATIONS
SEE PROFILE

DOI: 10.4255/mcpharmacol.11.04



# Evaluation of Anticancer Properties of Medicinal Plants from the Indian Sub-Continent

**Akbar Nawab[1], Mohammad Yunus[2], Abbas Ali Mahdi[3] and Sanjay Gupta[1,4,5]**

*Department of Urology, [1]Case Western Reserve University, [4]The Urology Institute, University Hospitals Case Medical Center, and [5]Case Comprehensive Cancer Center, Cleveland, Ohio; [2]Department of Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, India; [3]Department of Biochemistry, King George's Medical University, Lucknow, India.*

## Abstract

**Components of the plants viz. *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayta* have been used in traditional folk medicine to treat various human ailments; however, the anticancer properties have not been elucidated. We evaluated the anticancer properties of aqueous extracts of these plants against various human cancer cell lines. Exposure of aqueous extract of *Solanum nigrum* and *Artemisia vulgaris* exerted an inhibitory effect on cell growth and colony formation of the prostate, breast and colorectal cells. Other plant extracts exhibited a modest inhibition in cell proliferation for all three cell lines. These results were consistent with induction of apoptosis in cancer cells as measured by internucleosomal DNA fragmentation, caspase-3 activation and poly(ADP)ribose polymerase cleavage. Based on the in vitro data, it is suggested that consumption of the components of these plants or ingestion of extract as tea may impart anticancer effects especially in the colon, breast and the prostate.**

**Keywords:** Apoptosis; DNA fragmentation; Cell proliferation; Cancer; Indian plants

## Introduction

Carcinoma of the breast, colon and prostate are highly prevalent malignancies in the Western nations and accounts for approximately half of the total cancer-related deaths among men and women (1). The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management. Chemoprevention involves the use of pharmacological, dietary bio-factors, phytochemicals and even whole plant extracts to prevent, arrest, or reverse the cellular and molecular processes of carcinogenesis due to its multiple intervention strategies (2).

Natural phytochemicals derived from medicinal plants have gained significant recognition in the potential management of several human clinical conditions, including cancer (2-4). Much research has been geared towards the evaluation of plant extracts as prophylactic agents, which offer great potential to inhibit the carcinogenic process. Simultaneously, the synergistic effects of the cocktail of plant metabolites and the multiple points of intervention offer higher efficacy during chemoprevention regimens (4). The preventive mechanisms of tumor promotion by natural phytochemicals range from the inhibition of genotoxic effects, increased antioxidants and anti-inflammatory activity, inhibition of proteases and cell proliferation, protection of intracellular communications to modulate apoptosis and signal transduction pathways (5). A number of new chemopreventive agents are being identified based on the ability to modulate one or more specific molecular events. The discovery of effective herbs and elucidation of their underlying mechanisms could lead to the development of an alternative and complementary method for cancer prevention and/or treatment. The Indian sub-continent has great botanical diversity and widespread use of traditional medicine practice known as ayurvedic medicine; however, only a relatively small number of these plants have been subjected to accepted scientific evaluation for their potential anticancer effects (6). Based on an analysis of published literature, we

Received 02/22/11; accepted 02/25/11
Correspondence: Sanjay Gupta, Ph.D., Department of Urology, Case Western Reserve University, 2109 Adelbert Road, Wood Research Tower, Cleveland, Ohio 44106, USA. Tel. 216-368-6162. email: sanjay.gupta@case.edu

selected five traditional Indian plants with medicinal value to evaluate their anticancer efficacy, including: *Artemisia vulgaris* (Compositae), *Cichorium intybus* (Compositae or Asteraceae), *Smilax glabra* (Liliaceace), *Solanum nigrum* (Solanaceae) and *Swertia chirayta* (Gentinaceae).

*Artemisia vulgaris* is one of the several species in the genus Artemisia with names suffixing mugwort (7). Used for centuries as an alternative medicine, it possesses antibacterial, anthelmintic, anti-inflammatory, antiseptic, antispasmodic, carminative, digestive, diuretic, nervine, and purgative properties. It is traditionally used in alternative medicine to expel intestinal worms, nervous and spasmodic affections, asthma, sterility, functional bleeding of the uterus and menstrual complaints. The major constituents present in Artemisia sp. include tannins, polyphenols, flavonoids, saponosides, minerals and essential oils (7, 8)

The plant *Cichorium intybus* commonly known as Chicory or Kasni is used as a liver tonic, cardiotonic, diuretic, cholagogue, depurative, emmenagogue, in hepatomegaly, cephalalgia, inflammations, anorexia, dyspepsia, jaundice, splenomegaly, amenorrhea, and dysmenorrheal (9, 10). The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins (11).

Smilax plants particularly the tuber of *Smilax glabra* known as "Ba Qia" (or "Jin Gang Teng") in Chinese is used for the treatment of rheumatic arthritis, detoxification, lumbago, gout, and some inflammatory diseases (12, 13). Recent pharmacological investigations showed that Smilax has anti-inflammatory activity and the rhizome extract of *Smilax glabra* demonstrate immunomodulatory, free radical scavenging and antioxidant fortifying activities. Furthermore, some steroidal saponins isolated exhibited significant cytotoxicity activity against neoplastic cells (13, 14).

The plant *Solanum nigrum* commonly known as 'Back nightshade' has been extensively used in traditional medicine because of its diuretic and antipyretic effects, and has a long history of use in the treatment of inflammation, edema, mastitis, cirrhosis of liver in oriental medicine (15, 16). The whole plant of *Solanum nigrum* contains many steroidal glucosides, steroidal alkaloids, polyphenols, flavonoids, fatty acids and essential oils (17).

The plant *Swertia chirayta* commonly known as 'Chirata' or 'Kirata-tikta' is well known for its multifarious therapeutic values and is widely used as a crude drug (18). Among the different species of *Swertia* reported, *Swertia chirayata* is considered the most important for its medicinal properties. It possesses anti-helminthic, hypoglycemic, febrifuge, anti-malarial, anti-diarrheal and antipyretic properties which are attributed to amarogentin (the most bitter compound isolated), swerchirin, swertiamarin and other active compounds of the herb (19, 20). The phytochemical investigation of the genus *Swertia,* has identified more than 200 compounds with varying structural patterns. Among these constituents, the xanthanoids, terpenoids, flavonoids, alkaloids, irridoid and seco-irridoid glycosides form the major class (18-20).

In India, these herbal plants are commonly used in the treatment of various ailments and are also consumed regularly as a part of the daily diet. Despite their widespread use, however, no scientific assessment for anticancer effect has been conducted in most cases. Considering their increasing recognition and consumption, the present study was undertaken to evaluate the anticancer potential of these plant extracts in the inhibition of cell proliferation, colony formation and induction of cell death in human breast, colorectal and prostate cancer cells.

## Materials and Methods

### *Materials*

The herbal components of the plants were purchased from The State Unani Tibbya College, Lucknow, India. The plants were authenticated by the Department of Pharmacognosy, National Botanical Research Institute, Lucknow, India, where voucher specimens have been deposited. Antibodies of anti-caspase-3 and anti-PARP were purchased from Cell Signaling Technologies (Danvers, MA), and anti-β-actin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies for mouse and rabbit (horseradish peroxidase conjugates) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced chemiluminescence kit was purchased from GE Healthcare Biosciences (Piscataway, NJ). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) was purchased from Sigma (St. Louis, MO).

### *Preparation of extracts*

Based on an analysis of published literature the following plant parts were considered for the assessment

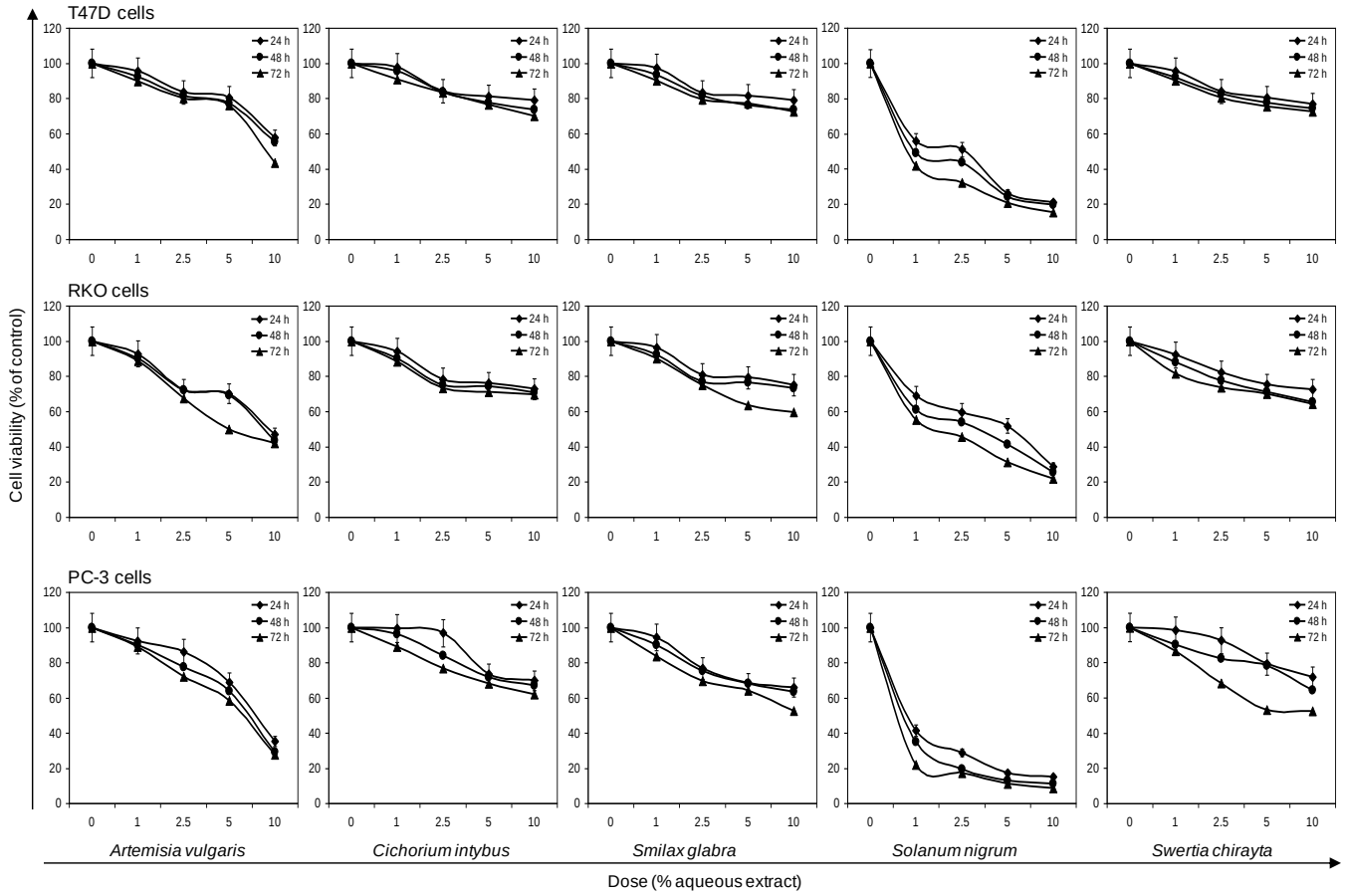


**Figure 1. Effect of aqueous extracts of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayata* on cell proliferation.** Human breast cancer T47D cells, colon carcinoma RKO cells and prostate cancer PC-3 cells were exposed to various concentrations of the above mentioned plant extracts for 24, 48 and 72 h, and the viability of the cells was determined by MTT assay. Cell viabilities are shown as percentages, and the untreated cells were regarded as 100% viable. Data represent the means of the three experiments conducted in triplicate. The details are described under 'Materials and Methods'.

of anticancer properties: inflorescence (*Artemisia vulgaris*), seeds (*Cichorium intybus*), rhizome (*Smilax glabra*), berries (*Solanum nigrum*) and whole plant (*Swertia chirayata*). Dry parts of these plants were weighed and crushed to powder with a marble mortar and pestle, and a 5% (w/v) suspension was prepared in a flask by adding distilled hot boiling water. The flask was then placed on a shaker (200 rpm) for 4 hours, and the temperature was maintained at 37°C, after which the flask was brought to room temperature and then the suspension was filtered through a series of Whatman filters and finally passed through a 0.22μm filter (Millipore, Billerica, MA).The filtered aqueous extract was lyophilized on a Speed Vac and a stock solution was then prepared by dissolving the extract powder in distilled water and the experimental concentrations were diluted in basal medium.

### *Cells*

Human prostate cancer PC-3 cells was cultured in RPMI 1640 medium with 5% FBS and 1% penicillin–streptomycin cocktail at 37 °C in a humidified atmosphere of 5% $CO_2$. Human breast carcinoma T47D cells and colon cancer RKO cells were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin–streptomycin cocktail.

### *Cell viability assay*

The effect of aqueous extract of the plants on the viability of cells was determined using MTT assay and growth inhibition was assessed as percent viability where vehicle treated cells was considered as 100% viable (21).

### *Colony formation assay*

Anchorage-independent colony formation assay was performed as previously described (22). Briefly,

cells at the initial density of 1x $10^5$ in 2 ml medium were seeded in 6-well plates containing 2 ml of 0.5% agar in medium as the bottom layer, 1 ml of 0.38% agar in medium. The cultures were incubated with 5% and 10% aqueous extract of *Artemisia vulgaris* and *Solanum nigrum* for 7 days. Cultures were maintained at $37^0$C in a humidified 5% $CO_2$ atmosphere. The number of colonies was determined by counting them under an inverted phase-contrast microscope at X400 magnification and a group of ~50 cells were counted as a colony.

### *Light microscopy*

Human breast, colon and prostate cancer cells were grown to 70% confluence and treated with 5% aqueous plant extracts for 24h and the photographs were taken at 40X magnification using a phase-contrast inverse microscope (Olympus, Japan).

### *DNA Fragmentation Assay*

Fragmentation of chromatin to units of single or multiple nucleosomes that form the nucleosomal DNA ladder in agarose gel is an established hallmark of programmed cell death or apoptosis. The cells grown to about 70% confluence and treated with 5% aqueous plant extracts for 48h and subjected to processing for DNA isolation and fragmentation assay as previously described (21, 22). The bands were visualized under an UV transilluminator, followed by digital photography.

### *Western blotting*

For Western blotting, 40µg proteins was resolved over 4-20% Tris-glycine polyacrylamide gel and then transferred onto the nitrocellulose membrane as previously described (22). The blots were blocked using 5% nonfat dry milk and probed using appropriate primary antibodies overnight at 4°C. The membrane was then incubated with appropriate secondary antibody horseradish peroxidase conjugate (Santa Cruz Biotech) followed by detection using chemiluminescence ECL kit (GE Healthcare Biosciences). For equal loading of proteins, the membrane was probed with appropriate loading controls.

### *Statistical Analysis*

The values are expressed as means ± SD. The significance between the control and treated groups was determined by Student's t test, and p values of less than 0.05 were taken to be significant in the experiments.

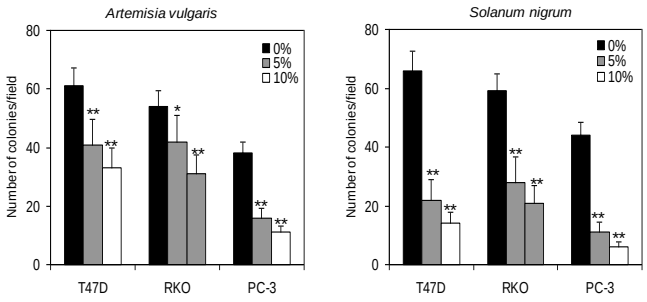


**Figure 2. Effect of aqueous extracts of *Artemisia vulgaris* and *Solanum nigrum* on anchorage-independent growth assay estimated by soft agar colony formation.** Human breast cancer T47D cells, colon carcinoma RKO cells and prostate cancer PC-3 cells were grown over 0.38% agar in medium along with specified concentration of the above mentioned plant extracts. The number of colonies was recorded after 7 days after treatment. Data represents the mean ± SD of three different assays. *p < 0.05, and **p < 0.001 *versus* control. The details are described under 'Materials and Methods'.

## Results

Herbal plants have been the basis for nearly all medicinal therapies since ancient times and the relevance of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayta* is well documented in the treatment of various human ailments in ayurvedic medicine (6, 23). Most of these therapies involve the use of plant extracts or their active phytochemical components, of which some have been shown to display anti-neoplastic activity (23, 24). The dry herbal parts of the plants which have been used for preparation of aqueous extract are described in 'materials and methods' section. To evaluate the effect of plants on cell proliferation, we investigated the effects of aqueous extracts of various plants on the growth and morphology of human breast cancer T47D cells, colon cancer RKO cells, and prostate cancer PC-3 cells, respectively. Cells were exposed to increasing doses of aqueous plant extracts for 24, 48, and 72 hours and cell viability was determined by the MTT assay. As shown in Figure 1, cell viability in all three cell lines was markedly decreased after exposure to *Artemisia vulgaris* and *Solanum nigrum* extract in dose-dependent manner. Other plant extracts obtained from *Cichorium intybus*, *Smilax glabra*, and *Swertia chirayta* demonstrated a modest decrease in cell growth inhibition in all three cancer cell lines. Aqueous extract obtained from berries of *Solanum nigrum* caused inhibition of 44-79% in T47D cells at the concentration ranging from 1.0 to 10% after 24 hours exposure. Similarly *Artemisia vulgaris* (inflorescence) demonstrated 5-42%,

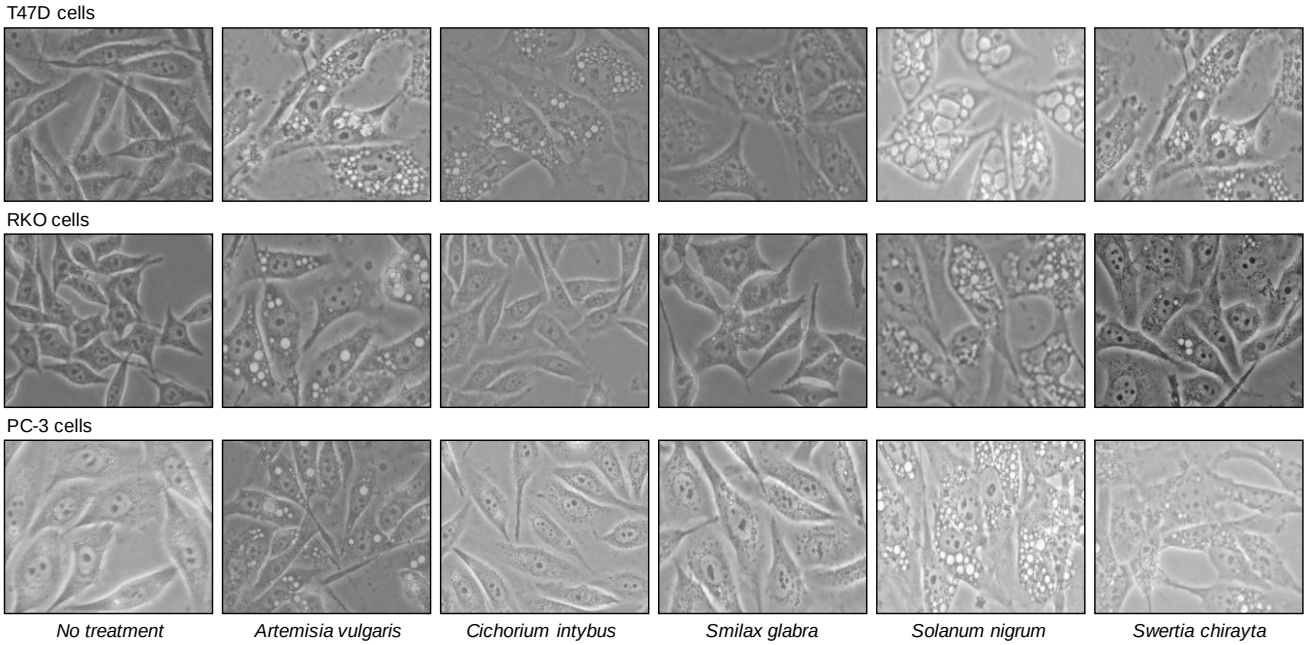


**Figure 3. Microscopic examination of human breast cancer T47D cells, colon carcinoma RKO cells and prostate cancer PC-3 cells after treatment with aqueous extracts of *Artemisia vulgaris, Cichorium intybus, Smilax glabra, Solanum nigrum* and *Swertia chirayata.*** The cells were treated with 5% aqueous extract of various plants for 24 h. Photographs were taken using an Olympus camera at a magnification of 40X.

*Cichorium intybus* (seeds) 2-21%, *Smilax glabra* (rhizome) 3-21% and *Swertia chirayta* (whole plant) exhibited 5-24% inhibition in cell viability at 1.0 to10% concentration for 24 hours. RKO cells exposed to *Solanum nigrum* demonstrated 31-76%, *Artemisia vulgaris* 7-53%, *Cichorium intybus* 6-26%, *Smilax glabra* 4-25%, and *Swertia chirayta* exhibited 8-28% inhibition at similar doses for the 24 hour treatment. Similarly, PC-3 cells exposed to *Solanum nigrum* demonstrated 59-85%, *Artemisia vulgaris* demonstrated 8-65%, *Cichorium intybus* 2-30%, *Smilax glabra* 6-34%, and *Swertia chirayta* exhibited 2-28% inhibition at similar doses for the 24 hour dosage. An increased cell growth inhibition was observed after incubation of these cells from 24 to 72 hours at various doses indicating that the aqueous plant extracts are cytotoxic to human cancer cells and are capable of exerting inhibition of cell proliferation.

Subsequently, we determined the effects of the aqueous extracts of *Artemisia vulgaris* and *Solanum nigrum* on colony formation. As evident in figure 2, treatment of human breast cancer T47D cells, colorectal cancer RKO cells and prostate cancer PC-3 cells with 5% and 10% aqueous extract of *Artemisia vulgaris* and *Solanum nigrum* resulted in a significant decrease in anchorage-independent growth and colony formation when compared with respective controls. A decrease of 41 and 33 colonies/field were noted in T47D cells, compared to control (61 colonies/field) after 5% and 10% exposure with extract of *Artemisia vulgaris*. A similar decrease in 42-31 colonies/field (control 54 colonies/field) and 16-11 colonies/field (control 38 colonies/field) were observed in RKO and PC-3 cells after treatment with *Artemisia vulgaris*. Aqueous extract of *Solanum nigrum* resulted in 22-14 colonies/field (control 66 colonies/field) in T47D cells, 28-21 colonies/field (control 59 colonies/field) in RKO cells and 11-6 colonies/filed (control 44 colonies/field) in PC-3 cells after treatment 5% and 10% aqueous extract.

Usually, cells undergoing apoptosis display a very similar pattern of morphological changes. These include blebbing, loss or cell membrane symmetry and attachment, cell shrinkage, nuclear fragmentation and chromatin condensation (25). As illustrated in figure 3, treatment of T47D, RKO and PC-3 cells with aqueous plant extracts exhibited typical features of apoptosis including surface bleb formation, redistribution and compaction of cytoplasmic organelles, formation of cytoplasmic vacuoles and hyperconvolution of the nuclear membrane. We observed that treatment with *Solanum nigrum* caused prominent changes in morphology in all the three cancer cell lines.

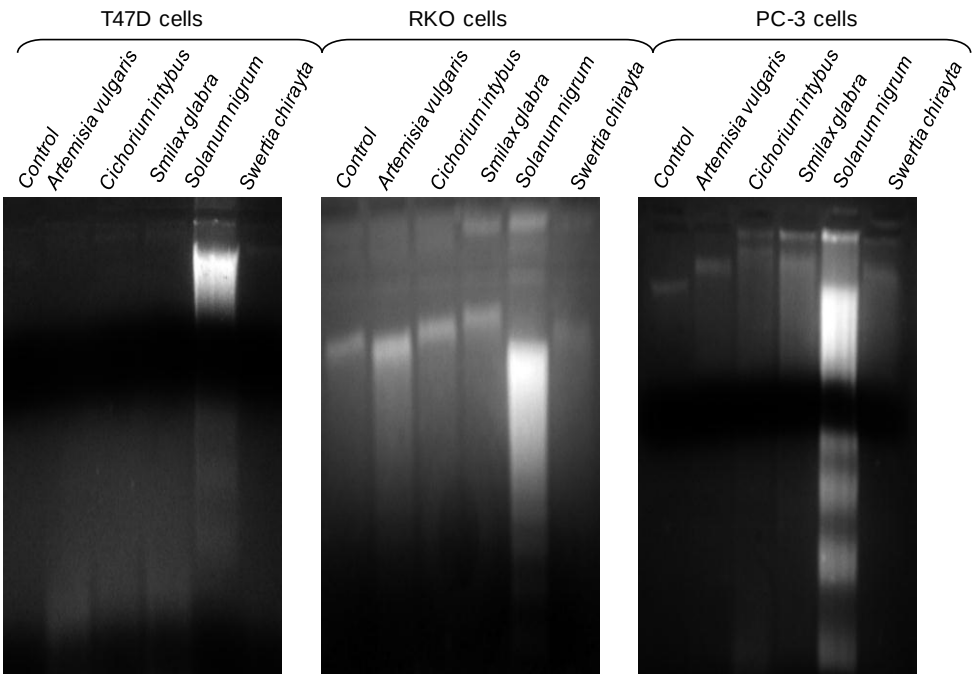


**Figure 4. Effect of aqueous extracts of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayata* on induction of apoptosis in various cancer cells.** Human breast cancer T47D cells, colon carcinoma RKO cells and prostate cancer PC-3 cells were exposed to 5% of the plant extracts for 48 h and DNA fragmentation assay was performed. The details are described under 'Materials and Methods'.

Furthermore, *Artemisia vulgaris*, *Cichorium intybus* and *Swertia chirayata* showed some effects in T47D cells, whereas *Smilax glabra* treatment did not show any significant morphological changes. In order to evaluate the apoptotic effect of the plant extracts, we performed DNA fragmentation assay in all the three cell lines after treatment with 5% aqueous extract of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayata* for 48 hours. These cells were processed for the DNA fragmentation assay. We observed that the extract of *Solanum nigrum* caused internucleosomal DNA fragmentation in all the three cell lines exhibiting the characteristic features of apoptosis. The intensity of fragmented DNA bands was prominent in cells treated with *Solanum nigrum* when compared to other aqueous plant extracts that exhibited a decreased or no intensity (Figure 4).

Apoptosis is a consequence of a highly complex cascade of cellular events, and caspase-3 has been implicated in the execution phase of apoptosis cleaving over 100 substrates, including poly(ADP-ribose)polymerase (PARP) (26, 27). We determined the protein expression of cleaved PARP at Asp214 that detects endogenous levels of the large fragment (89 kDa) of human PARP1 produced by caspase cleavage. Furthermore, we determined the cleaved caspase-3 that detects endogenous levels of the large fragment (17/19 KDa) of activated caspase-3 resulting from cleavage adjacent to Asp175 after exposure of cells with aqueous plant extracts. As depicted in figure 5, the 85-kDa cleavage product of PARP was evident in all 3 human cancer cells treated with various extracts. In PC-3 cells, 5 and 10% of aqueous extracts of *Artemisia vulgaris*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayata* resulted in the cleaved PARP product, whereas 10% extract of *Cichorium intybus* caused PARP cleavage. In RKO cells, all the extracts at 5 and 10% exhibited PARP cleavage and in the breast cancer T47D cells aqueous extracts of *Artemisia vulgaris*, *Cichorium intybus Smilax glabra*, *Solanum nigrum* caused a dose-dependent increase in cleaved PARP. Consistently, treatment with the plant extracts caused cleavage in caspase-3 indicating that the aqueous plant extracts has potential to induce apoptosis in various human cancer cells.

## Discussion

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world (2-5). Approximately,

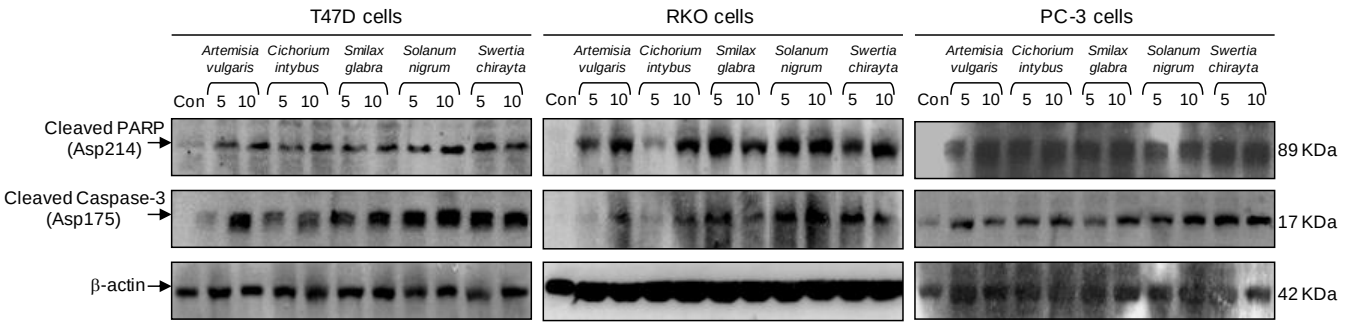


**Figure 5. Effect of aqueous extracts of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayata* on the protein expressions of cleaved PARP (Asp214) and cleaved Caspase-3 (Asp175) in cancer cells.** Human breast cancer T47D cells, colon carcinoma RKO cells and prostate cancer PC-3 cells were exposed to various concentrations of the above mentioned plant extracts for 24 and the samples were subjected to electrophoresis by SDS-PAGE. The blots were analyzed with the indicated antibodies and β-actin was used as the loading control. The details are described under 'Materials and Methods'.

60% of the anticancer drugs currently used have been isolated from natural products from the plants. At this time, more than 3000 plants worldwide have been reported to possess anticancer properties (28). The parts of plants viz. inflorescence (*Artemisia vulgaris*), seeds (*Cichorium intybus*), rhizome (*Smilax glabra*), berries (Solanum nigrum) and whole plant (*Swertia chirayata*) have been commonly used in traditional Indian medicine for the treatment of various human ailments for many years (23, 24). Extracts of these medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and/or therapeutic properties (28). Our goal was to determine whether the extracts of these plants exerted an inhibitory effect on cancer cell proliferation and caused cell death. The results of our studies suggest that aqueous extract of *Solanum nigrum* and *Artemisia vulgaris* possess the strongest cytotoxic effects on various human cancer cells. The moderate anticancer activity was observed in *Cichorium intybus*, *Smilax glabra*, and *Swertia chirayta.*

The initial screening of plants for their anticancer properties use cell-based assays and established cell lines, in which the cytotoxic effects of plants extracts or isolated compounds could be measured. In the present study, we used MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay and the colony formation assay to detect the anti-proliferative activity of plant extracts *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayta* in three different human cancer cells. We observed that the extract of *Artemisia vulgaris* caused marked cell growth inhibition in the human prostate cancer PC-3 and breast cancer T47D cells in a dose- and time-dependent manner. Moreover, the same doses were effective in human colon cancer cells at 72 hours. The whole plant extract obtained from *Artemisia vulgaris* revealed the presence of tannins, polyphenols, flavonoids, such as eupafolin, diosmetin, rhamnetin, apigenin and their glucosides, luteolin, quercetin, rutin and vitexin which are characterized for their anticancer properties. Extracts of this species also contains many essential oils such as terpenes and terpene derivatives, viz., 1, 8 cineol, camphor, linalool, thujone, 4-terpineole, borneol, α––cardinol, mono- and sesquiterpenes (7, 8). Constituents derived from Artemisia sp. viz. artemisinin have been approved for use in humans as an anti-malarial drug. Hence, unraveling its anticancer properties and underlying mechanisms will be critical to determine which cancer phenotype can best be treated with this phytochemical and for testing and pharmacological characterization in humans (8, 29). Another study has reported that constituents isolated from Artemisia vestita exhibited marked anticancer property in inhibiting human pancreatic cancer cell proliferation (30).

The aqueous extract of *Solanum nigrum* was observed to be the most effective in cell growth inhibition for all the three cancer cells. The whole plant extract of *Solanum nigrum* has been shown to contain many steroidal glycosides, steroidal alkaloids, and steroidal oligoglycosides, including solamargine, solasonine, solavilline, solasdamine, and solanine. Besides, it has been reported to contain many polyphenolic compounds such as gallic acid, protocatechuic acid, catechin, caffeic acid, epicatechin, rutin, and naringenin, which possess strong antioxidant and anticancer activity (16, 17, 28). Previous studies investigated the effect of *Solanum nigrum* in skin cancer cells, which resulted

in inhibition in cell migration and invasion (31). In another study, aqueous powdered extract of *Solanum nigrum* Lynn caused apoptosis and autophagy in human liver cancer HepG2 cells (32). It is suggested that the pronounced anti-proliferative activities of *Solanum nigrum* extracts on various human cancer cell lines is critical to uncover the target proteins and to determine the precise cellular pathways by which the Solanum compounds mediate their cancer-specific effects.

Apoptosis or programmed cell death involves a sequential cascade of cellular event, resulting from chromatin condensation, DNA fragmentation, cytoplasmic membrane blebbing and cell shrinkage (25). This involves various initiator and executor caspases. The executor caspase, caspase-3 has been reported to cleave a number of substrates including PARP which act in response to DNA strand breaks leading to apoptosis (26, 33). PARP cleavage occurs at Asp216 to generate 85 and 31 KDa apoptotic fragments during apoptosis (26, 27). We investigated whether these plant extracts can induce caspase-3 activation and result in PARP cleavage in the breast, prostrate and colorectal cancer cells. Results from the present study indicate that all the plant extracts caused caspase-3 activation that resulted in PARP cleavage. However, the effect was more pronounced in the aqueous extracts of *Solanum nigrum* and *Artemisia vulgaris*.

We studied the cytotoxic effect of plant extracts from *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayta* in various human cancer cell lines of the prostrate, breast and colorectum. We used multiple techniques to confirm the anti-proliferative and apoptotic effects of these aqueous extracts in cancer cells. Our study provides evidence that aqueous extracts of *Artemisia vulgaris*, *Cichorium intybus*, *Smilax glabra*, *Solanum nigrum* and *Swertia chirayta* have the ability to inhibit cell proliferation, colony formation and induce apoptosis in cancers of breast, colorectal and prostrate, albeit at different dose levels. In conclusion, the in vitro data presented here suggests that consumption of the parts of these plants or ingestion of extract as tea may impart anticancer effects. Further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition which will allow the rationale design for more effective molecules for the eventual use as cancer chemopreventive and/or therapeutic agents.

## Acknowledgements

This work was supported by Endowment grant to Sanjay Gupta.

## Conflicts of Interest

No potential conflicts of interest to disclose.

## References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come? Pharm Res 2010;27:950-61.
3. Desai AG, Qazi GN, Ganju RK, et al. Medicinal plants and cancer chemoprevention. Curr Drug Metab 2008;9:581-591
4. Guilford JM, Pezzuto JM. Natural products as inhibitors of carcinogenesis. Expert Opin Investig Drugs 2008;17:1341-52.
5. Soobrattee MA, Bahorun T, Aruoma OI. Chemopreventive actions of polyphenolic compounds in cancer. Biofactors 2006;27:19-35.
6. Krishnaswamy K. Traditional Indian spices and their health significance. Asia Pac J Clin Nutr 2008;17:265-8.
7. Tan RX, Zheng WF, Tang HQ. Biologically active substances from the genus Artemisia. Planta Med 1998;64:295-302.
8. Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from Artemisia annua L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 2010;15:3135-70.
9. Schaffer S, Schmitt-Schillig S, Müller WE, Eckert GP. Antioxidant properties of Mediterranean food plant extracts: geographical differences. J Physiol Pharmacol 2005;56:115-24.
10. Tousch D, Lajoix AD, Hosy E, et al. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. Biochem Biophys Res Commun 2008;377:131-5.
11. Innocenti M, Gallori S, Giaccherini C, Ieri F, Vincieri FF, Mulinacci N. Evaluation of the phenolic content in the aerial parts of different varieties of *Cichorium intybus* L. J Agric Food Chem 2005;53:6497-6502.
12. Ooi LS, Wong EY, Chiu LC, Sun SS, Ooi VE. Antiviral and anti-proliferative glycoproteins from the rhizome of *Smilax glabra* Roxb (Liliaceae). Am J Chin Med 2008;36:185-95.
13. Shao B, Guo HZ, Cui YJ, et al. Simultaneous determination of six major stilbenes and flavonoids in Smilax china by high performance liquid chromatography. J Pharm Biomed Anal 2007;44:737-42.
14. Spelman K, Burns J, Nichols D, Winters N, Ottersberg S, Tenborg M. Modulation of cytokine expression by traditional medicines: a review of herbal immunomodulators. Altern Med Rev 2006;11:128-50.
15. Jainu M, Devi CS. Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. J Ethnopharmacol 2006;104:156-63.

16. Heo KS, Lim KT. Antioxidative effects of glycoprotein isolated from *Solanum nigrum* L. J Med Food 2004;7:349-57.
17. Lin HM, Tseng HC, Wang CJ, Lin JJ, Lo CW, Chou FP. Hepatoprotective effects of *Solanum nigrum* Linn extract against CCl(4)-induced oxidative damage in rats. Chem Biol Interact 2008;171:283-93.
18. Brahmachari G, Mondal S, Gangopadhyay A, et al. *Swertia* (Gentianaceae): chemical and pharmacological aspects. Chem Biodivers 2004;1:1627-51.
19. Kumar IV, Paul BN, Asthana R, Saxena A, Mehrotra S, Rajan G. *Swertia chirayita* mediated modulation of interleukin-1beta, interleukin-6, interleukin-10, interferon-gamma, and tumor necrosis factor-alpha in arthritic mice. Immunopharmacol Immunotoxicol 2003;25:573-83.
20. Suryawanshi S, Mehrotra N, Asthana RK, Gupta RC. Liquid chromatography/tandem mass spectrometric study and analysis of xanthone and secoiridoid glycoside composition of *Swertia chirata*, a potent antidiabetic. Rapid Commun Mass Spectrom 2006;20:3761-8.
21. Srivastava JK, Gupta S. Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. J Agric Food Chem 2007;55:9470-8.
22. Shukla S, Gupta S. Molecular mechanisms for apigenin-induced cell-cycle arrest and apoptosis of hormone refractory human prostate carcinoma DU145 cells. Mol Carcinog 2004;39:114-26.
23. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharmacol 2006;103:25-35.
24. Singh B, Bhat TK, Singh B. Potential therapeutic applications of some antinutritional plant secondary metabolites. J Agric Food Chem 2003;51:5579-97.
25. Bøe R, Gjertsen BT, Vintermyr OK, Houge G, Lanotte M, Døskeland SO. The protein phosphatase inhibitor okadaic acid induces morphological changes typical of apoptosis in mammalian cells. Exp Cell Res 1991;195:237-46.
26. Mancini M, Nicholson DW, Roy S, et al. The caspase-3 precursor has a cytosolic and mitochondrial distribution: implications for apoptotic signaling. J Cell Biol 1998;140:1485-95.
27. Soldani C, Scovassi AI. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 2002;7:321-8.
28. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010;15:7313-52.
29. Firestone GL, Sundar SN. Anticancer activities of artemisinin and its bioactive derivatives. Expert Rev Mol Med 2009;11:e32.
30. He L, Wu Y, Lin L, et al. Hispidulin, a small flavonoid molecule, suppresses the angiogenesis and growth of human pancreatic cancer by targeting vascular endothelial growth factor receptor 2-mediated PI3K/Akt/mTOR signaling pathway. Cancer Sci 2011;102:219-25.
31. Wang HC, Wu DH, Chang YC, Li YJ, Wang CJ. *Solanum nigrum* Linn. water extract inhibits metastasis in mouse melanoma cells in vitro and in vivo. J Agric Food Chem 2010;58:11913-23.
32. Lin HM, Tseng HC, Wang CJ, et al. Induction of autophagy and apoptosis by the extract of *Solanum nigrum* Linn in HepG2 cells. J Agric Food Chem 2007;55:3620-8.
33. Nicholson DW, Thornberry NA. Caspases: killer proteases. Trends Biochem Sci 1997;22:299-306.