DOI:http://dx.doi.org/10.7314/APJCP.2015.16.15.6423

RESEARCH ARTICLE

In Vitro Anticancer Activities of *Anogeissus latifolia*, *Terminalia bellerica*, *Acacia catechu* and *Moringa oleiferna* Indian Plants

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Abstract

The present study was designed to evaluate *in vitro* anti-proliferative potential of extracts from four Indian medicinal plants, namely *Anogeissus latifolia*, *Terminalia bellerica*, *Acacia catechu* and *Moringa oleiferna*. Their cytotoxicity was tested in nine human cancer cell lines, including cancers of lung (A549), prostate (PC-3), breast (T47D and MCF-7), colon (HCT-16 and Colo-205) and leukemia (THP-1, HL-60 and K562) by using SRB and MTT assays. The findings showed that the selected plant extracts inhibited the cell proliferation of nine human cancer cell lines in a concentration dependent manner. The extracts inhibited cell viability of leukemia HL-60 and K562 cells by blocking G0/G1 phase of the cell cycle. Interestingly, *A. catechu* extract at 100 μg/mL induced G2/M arrest in K562 cells. DNA fragmentation analysis displayed the appearance of a smear pattern of cell necrosis upon agarose gel electrophoresis after incubation of HL-60 cells with these extracts for 24h.

Keywords: Anogeissus latifolia - terminalia bellerica - acacia catechu - moringa oleiferna - cytotoxicity - DNA ladder

Asian Pac J Cancer Prev, **16 (15)**, 6423-6428

Introduction

There has been a long-standing interest in identification of natural products and medicinal plants for developing new cancer therapeutics. India, represented by rich culture, traditions, and natural biodiversity, offers a unique opportunity for researchers in drug discovery and development (Brusotti et al., 2014). This country has 2 of 18 hotspots of plant biodiversity in the world, namely Eastern Himalaya and Western Ghats (Chitale et al., 2014). In the present study, we selected four medicinal plants namely *Anogeissus latifolia*, *Terminalia bellerica*, *Acacia catechu* and *Moringa oleiferna* to explore their anticancer efficacy in human cancer cell lines. Stem bark of *Anogeissus latifolia* has been extensively utilized in the treatment of various disorders like skin diseases, snake and scorpion bites, leprosy, diabetes, stomach diseases, colic, cough, and diarrhea (Patil and Gaikwad, 2011). The fruit of *Terminalia bellerica* has been used for the treatment of anemia, asthma, cancer, colic, constipation, diarrhea, dysuria, headache, hypertension, inflammation, and rheumatism (Rashed et al., 2014). The bark and heartwood of *Acacia catechu* are widely used for the treatment of chronic fever, ulcer, cough, worm infestation, poisonous bites, obesity, hepatomegaly, spleeno¬megaly, and problems related to skin, throat, tooth, and urinary tract (Stohs and Bagchi, 2015). Various parts of *Moringa oleifera* such as leaves, roots, seed, bark, fruit, flowers and immature pods are used as cardiac and circulatory stimulants, and have been shown to have diuretic, antitumor, anti-inflammatory, antispasmodic, antibacterial, and antifungal activities (Caceres et al., 1992; Biswas et al., 2012; Krishnamurthy et al., 2015). Considering the vast therapeutic potential of above mentioned medicinal plants, the present study was planned to investigate their anti-proliferative potential in different panel of human cancer cell lines. To get some insight into the cellular mechanism of action of the extracts, the most active concentrations of the plants extracts were also studied for cell cycle arrest and apoptotic potential in human leukemia cell line.

Materials and Methods

Chemicals and reagents

The RPMI-1640 medium, Dulbecco's modified eagle's medium (DMEM), fetal calf serum (FCS), trypsin, gentamycin, penicillin, sulforhodamine blue (SRB), 3-(4, 5-dimethy- lthiazol-2-yl)-2, 5-diphenyl)-tetrazolium bromide (MTT), ethidium bromide, propidium iodide (PI), DNase-free RNase, proteinase K, dimethyl sulphoxide (DMSO), camptothecin, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), tris-base and phosphate buffered saline (PBS) were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Trichloroacetic acid (TCA) was procured from Merck Specialties Pvt. Ltd., (Mumbai, India).

Extraction Process

The plants were harvested from herbal garden of Indian Institute of Integrative Medicine (IIIM), Jammu,

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India. The plants were identified and authenticated by taxonomist of the IIIM. The extraction process of these plants was procured from the National products Chemistry Division of the IIIM. Dried and powdered plants were placed in a conical glass percolator, submerged in 95% ethanol or 50 % ethanol, and kept at room temperature for 20h (Table 1). The extraction procedure was repeated four times and the percolate were collected and filtered. Ethanol was distilled off from pooled percolate using a rotavapour under reduced pressure at 50°C. The final drying was done initially in vacuum desiccators and finally lyophilized. The dried extracts were scrapped off and transferred to a wide mouth glass container. Nitrogen was blown in the container before capping and stored at -20°C in a desiccator.

Cell culture and treatment

Nine human cancer cell lines were obtained from National Cancer Institute, Frederick, USA. The cell lines were derived from different cancers including lung (A549), prostate (PC-3), breast (T47D and MCF-7), colon (HCT-16, Colo-205), and leukemia (THP-1, HL-60 and K562). All cell lines were routinely cultured in RPMI-1640 growth medium except MCF-7 cell line was cultured in DMEM medium. Both growth media (pH 7.2) were supplemented with 10% FCS, 1% penicillin (100 U/mL) and streptomycin (10 mg/mL), in tissue culture flask in an incubator at 37°C with 95% relative humidity and 5% CO_2 gas environment. The cells were harvested either by trypsinization (adherent cultures) or by centrifugation at 1000 rpm for 5 min (suspension cultures). Stock solutions (20 mg/mL) of the extracts were dissolved in DMSO and serially diluted with complete growth medium containing 50 μg/mL of gentamycin to the desired concentrations (10, 30, 50, 70 and 100 μg/mL). Untreated control cultures received only the vehicle (DMSO<1%).

In vitro cytotoxicity screening

Sulforhodamine blue (SRB) assay: Measurement of the cellular protein content was performed using the SRB assay as described earlier (Vichai and Kirtikara, 2006). Briefly, seven adherent cultures namely A459, PC3, MCF-7, T47D, Colo-205, HCT-16 and THP-1 were harvested in log phase using trypsinization (0.05% trypsin and 0.02% EDTA, in PBS) and the cells were counted using a hemocytometer. The cells were seeded into 96-well plates at density 1000 cells/100µL/well excepting Colo-205 cells were seeded at density 1500 cells/well in 100µL medium into 96 well plate. After 24h, the medium was aspirated and the cells were exposed to 100 µL/well of freshly prepared medium containing test materials at desired concentrations for 48 h. At the end of exposure time, 50 µL of ice-cold 50% TCA was added to each well and left at 4°C for 1h to fix the cells attached to the bottom of the wells. The plates were washed five times with distilled water and then air-dried. The TCA-fixed cells were stained with SRB (0.4% in 1% acetic acid, 100 µL) for 30 min, followed by washing with 1% acetic acid and air-dried. The adsorbed SRB was dissolved by adding 100 µL of 10 mmol/L Tris buffer (pH 10.5) to each well and the plate was gently stirred for 10 min on a shaker platform. The absorbance at wavelength 540 nm was read using a microplate reader (Tecan, Switzerland).

MTT assay

Mitochondrial activity was evaluated by MTT assay as described earlier (Vega-Avila and Pugsley 2011). This assay based on enzymatic reduction of the yellow colored MTT dye to purple colored formazan crystals by a variety of mitochondrial and cytosolic enzymes that are operational in viable cells. Briefly, HL-60 and K562 cells (5000 cells/well) were seeded in 100 μL of medium into 96-well plate and left to settle in a CO_2 incubator. After 60 min of incubation, the test material was added in each well (100 µL/well) and the plate was incubated for 48 h. Four hours before the end of incubation period, 20 µL of MTT solution (2.5 mg/mL in PBS) was added to each well and re-incubated for 4h at 37°C. The plate was centrifuged with rotor for 96-well plate assembly (Beckman GS-6R, USA) at 3000 rpm for 15 min. Then, the supernatant culture medium containing MTT was removed and 200 µL of DMSO were added to each well to dissolve the formazan crystals. The optical density (OD) of each well was recorded using a microplate reader at a wavelength of 570 nm. The percentages of cell viability and growth inhibition were calculated according to the following equations (Chanda et al., 2012). Cell viability (%)=[(OD of treated cells-OD of blank)/ (OD of control-OD of blank)×100]. Growth inhibition (%)100 – % Cell viability

Cell cycle analysis

Flow cytometry was used to analyze cell cycle distribution according to the standard procedures (Saxena et al., 2010). Briefly, HL-60 and K562 cells (2×10^6 / mL/6 well plate) were treated with plant extracts at 50 and 100 µg/mL for 24 h. The cells were harvested and centrifuged at 400g for 5 min. The supernatant was discarded and the pellet was washed twice with 2 mL of PBS. The cells were fixed overnight in chilled 70% ethanol at 4°C and then subjected to RNase digestion (400 µg/mL) at 37°C for 1h. Finally, the cells were stained with PI (10 µg/mL) for 30 min in dark and analyzed immediately for DNA contents on a Flow Cytometer FACS Diva (Becton Dickinson, Franklin Lakes, NJ, USA). The cell cycle histograms were analyzed using the ModFit LT™ 3.2.1 software packages (Verity Software House Inc., Topsham, ME). In this program, debris and single cell populations were gated out using two parameter histogram of FL2-A versus FL2-W.

DNA ladder assay

HL-60 cells (2×10^6 cells/mL in 6-well plate) were harvested after 24h treatment with the selected plant extracts at 50 and 100 µg/mL. The cells were centrifuged at 1000 rpm for 10 min and washed in with PBS containing 20 mmol/L EDTA. The pellet was lysed in 250 µL of lysis buffer (100 mmol/L NaCl, 5 mmol/LEDTA, 10 mmol/L Tris-HCl, pH 8.0, and 5% Triton X-100) containing 400 μg/mL DNase-free RNase and incubated at 37°C for 90 min, followed by incubation with proteinase K (200 μg/mL) at 50°C for 2h. The DNA was extracted with phenol-chloroform-isoamyl alcohol (25: 24: 1) for 1 min and centrifuged at 15000 rpm for 2 min. The aqueous phase was further extracted with chloroform-isoamyl alcohol (24: 1) and centrifuged at 15000 rpm for 2 min. The DNA was allowed to precipitate with 3 volumes of chilled alcohol and 0.3mol/L sodium acetate at 20°C overnight. The precipitate was centrifuged at 15000 rpm for 10 min. The DNA pellet was washed in with 80% alcohol, dried, dissolved in 50 μL of Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.4) and subjected to electrophoresies in 1.8 % agarose gel at 50 V for 1.5h (Saxena et al., 2012).

Data analysis

All experiments were repeated at least three times, and each experimental condition was repeated at least in quadruplicate wells in each experiment. The IC_{50} values of plant extracts were calculated by linear regression analysis. All the data were analyzed by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for comparison between different treatment groups. Differences were considered statistically significant at $P<0.05$. All computations were made by employing the statistical package for Social Sciences software (SPSS Inc., version 17, Chicago, IL, USA).

Results

In vitro cytotoxicity

The anti-proliferative activity of the selected plant extracts was measured using the SRB assay for adherent cultures and MTT assay for suspension cultures against a panel of nine human tumor cell lines. The cytotoxicity assays were assessed at 48h following treatment with different concentrations of plant extracts in the range of 10-100 μg/mL. The findings showed that, the extracts inhibited differential growth inhibition against the examined cancer cells in a concentration dependent manner.

The sensitivity of the cell lines to extracts exposure was characterized by IC_{50} values (Table 2). In the US National Cancer Institute plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value in cancer cells is ≤30 μg/mL following incubation between 48-72h (Boik, 2001). According to this criterion, the extract of *A. Latifolia* was strongly active against A549, PC3, MCF-7, HCT-16, Colo-205, THP-1, and HL-60 cells (IC_{50}=10.6-28.7 μg/mL), and moderately active against T47D and K562 cells (IC_{50}=42.2-46.6 μg/mL). The extract of *T. bellerica* exerted strong cytotoxicity against A549, PC3, MCF-7, HCT-16, Colo-205, and THP-1 (IC_{50}=9.0-28.4 μg/mL) and moderate activity against T47D, HL-60, and K562 cells (IC_{50}=33.5-50.0 μg/mL). The extract of *A. catechu* was strongly active against A549, PC3, MCF-7, HCT-16, and HL-60 cells (IC_{50}=9.7-25.9 μg/mL) and moderately active against T47D, Colo-205, THP-1, and K562 cells (IC_{50}=37.8-42.8 μg/mL). The extract of *M. oleiferna* was strongly active against A549, PC3, MCF-7, and HCT-16 cells (IC_{50}=13.2-28.8 μg/mL) and moderately active against T47D, Colo-205, THP-1, HL-60, and K562 cells (IC_{50}=33.5-50.0 μg/mL).

Cell cycle analysis

Flow cytometry analysis exhibited an increase in the percentage of cells in G_0/G_1 phase with concomitant decrease in S-phase population in HL-60 and K562 cells treated with plant extracts (Figures 1&2). This implied that the extracts inhibited the proliferation of HL-60 and K562 cells through arresting G_0/G_1 cell cycle phase progression.

The sub-G_0/G_1 peak was increased to maximum values 37.21% in HL-60 and 17.49% in K562 cells treated with 100 μg/mL of extracts of *T. bellerica* and *A. Latifolia*, respectively vs 2.08% and 2.97% in the control HL-60 and K562 cells, respectively (Figures 1 and 2). However, insignificant change in the sub-G_0/G_1 fraction was observed in K562 cells treated with *A. Latifolia*, *T. bellerica* and *M. oleifera* indicating absence of apoptosis within this experimental duration (Figure 2).

Surprisingly, treatment of K562 cells with *A. catechu* at 100 μg/mL caused an increase in the G2/M peak (12.18% *vs* 8.80% in the control cells). Further G2/M

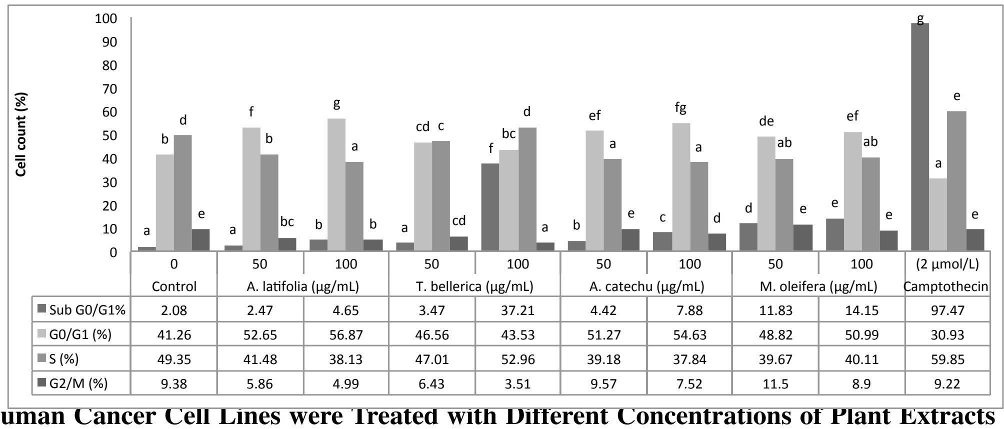
Table 1. Taxonomical Profile of Four Indian Medicinal Plants Used in the Current Study

Name of Plants	Family	Accession No.	Parts used	Extraction mode
Anogeissus Latifolia	Combretaceae	19909	Stem and leaves	95% EtOH extract
Terminalia bellerica	Combretaceae	17983	Stem bark	95% EtOH extract
Acacia catechu	Leguminosae	2901	Fruit	50%EtOH extract
Moringa oleifera	Moringaceae	20402	Leaves	50% EtOH extract

Table 2. IC_{50} Values (μg/mL) of Extracts of Four Indian Medicinal Plants Against Human Cancer Cell Lines

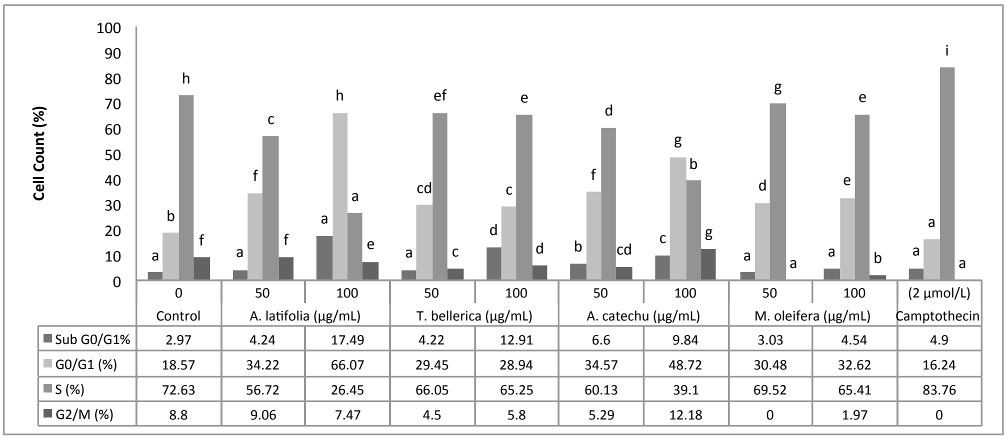
Cell lines	Anogeissus latifolia	Terminalia bellerica	Acacia catechu	Moringa oleiferna
A549	20.0±1.2	28.4±2.7	25.9±1.5	13.2±1.8
PC3	10.6±0.4	17.7±1.7	14.3±1.6	22.2±4.9
T74D	42.2±1.4	43.7±2.2	38.5±1.4	33.5±2.5
MCF-7	20.1±4.8	9.0±1.7	22.8±4.9	26.4±5.7
HCT-16	25.8±1.5	24.8±1.3	20.6±3.9	28.8±2.2
Colo-205	16.1±0.8	22.9±1.1	39.8±2.9	49.7±0.8
THP-1	21.0±2.4	9.6±1.9	37.8±2.6	35.8±1.7
HL-60	28.7±2.3	33.5±1.0	9.7±2.2	50.0±1.0
K562	46.6±2.4	50.0±1.9	42.8±2.6	49.9±1.7

*Human cancer cell lines were treated with different concentrations of plant extracts in 96-well microculture plates for 48h. IC_{50} values were determined by linear regression analysis using SPSS software



	0	50	100	50	100	50	100	50	100	(2 μmol/L)
	Control	A. latifolia (μg/mL)		T. bellerica (μg/mL)		A. catechu (μg/mL)		M. oleifera (μg/mL)		Camptothecin
■ Sub G0/G1%	2.08	2.47	4.65	3.47	37.21	4.42	7.88	11.83	14.15	97.47
■ G0/G1 (%)	41.26	52.65	56.87	46.56	43.53	51.27	54.63	48.82	50.99	30.93
■ S (%)	49.35	41.48	38.13	47.01	52.96	39.18	37.84	39.67	40.11	59.85
■ G2/M (%)	9.38	5.86	4.99	6.43	3.51	9.57	7.52	11.5	8.9	9.22

Figure 1. Human Cancer Cell Lines were Treated with Different Concentrations of Plant Extracts in 96-well Microculture Plates for 48h. IC_{50} values were determined by linear regression analysis using SPSS software. The different letters above the bars for each cell cycle phase indicate significant differences ($P < 0.05$) between treated cells as determined by ANOVA followed by Duncan's multiple comparison tests



	0	50	100	50	100	50	100	50	100	(2 μmol/L)
	Control	A. latifolia (μg/mL)		T. bellerica (μg/mL)		A. catechu (μg/mL)		M. oleifera (μg/mL)		Camptothecin
■ Sub G0/G1%	2.97	4.24	17.49	4.22	12.91	6.6	9.84	3.03	4.54	4.9
■ G0/G1 (%)	18.57	34.22	66.07	29.45	28.94	34.57	48.72	30.48	32.62	16.24
■ S (%)	72.63	56.72	26.45	66.05	65.25	60.13	39.1	69.52	65.41	83.76
■ G2/M (%)	8.8	9.06	7.47	4.5	5.8	5.29	12.18	0	1.97	0

Figure 2. The Cell Cycle Phase Distribution of Four Indian Medicinal Plants in K562 Cells Within 24h. The different letters above the bars for each cell cycle phase indicate significant differences ($P<0.05$) between treated cells as determined by ANOVA followed by Duncan's multiple comparison tests

phase was not affected in HL-60 cells treated with these extracts. Camptothecin was used as a positive control in this experiment and exhibited an increase in S-phase population in both HL-60 cells (59.85% *vs* 49.35% in the control) and K562 cells (83.76% *vs* 72.63% in the control)). It is worth noting that camptothecin exhibited an increase in sub-G_0/G_1 of HL-60 (97.47% *vs* 2.08% in control cells) and K562 (4.90% vs 2.97% in control cells).

DNA ladder assay

To elucidate whether plant extracts decreased cell survival by the induction of DNA fragmentation, genomic DNA was isolated from control and treated HL-60 cells and then subjected to 1.8% agarose gel electrophoresis. In the present study, no DNA ladder was observed in control or treated HL-60 cells with extracts (Figure 3). This implied that, incubation HL-60 treated with the extracts for 24h induced cell death which apparently was accompanied by the formation of a large DNA fragments, represented as smear upon agarose gel electrophoresis. On the other hand, a characteristic ladder of nucleosome-sized DNA fragments was observed in camptothecin-treated HL-60 cells.

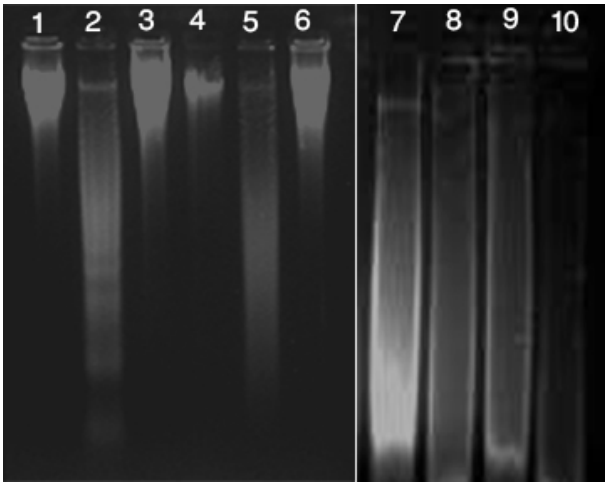


Figure 3. DNA Fragmentation Assay in HL-60 Cells. HL-60 cells (2×10^6/ml/well) were incubated with plant extracts for 24h. Lane 1 (untreated cells), lane 2 (2μM camptothecin), lanes 3-4 (50 & 100μg/ml of *A. latifolia*,), lanes 5-6 (50&100μg/ml of *T. bellerica*), lane 7-8(50&100μg/ml of *A. catechu*), lane 9-10 (50&100μg/ml of *M. oleiferna*)

Discussion

Our *in vitro* experiments showed that the selected plant extracts exhibited variety of cytotoxicity against the examined cancer cell lines. Among the examined cell lines, K562 cells were the most resistant toward the extracts with IC_{50} value in the range of 42.8-50 μg/mL. Whereas, PC3, MCF-7 and A459 are the most sensitive cells with

IC_{50} values in the range of 10.6-22.2 μg/mL, 9.0-26.4 μg/mL and 13.2-28.4 μg/mL, respectively. Such variation in cytotoxicity from one cell to another is due to that cancer cells posses differences in their origin, morphology and genomes, resulting in susceptibility difference to chemotherapeutic agents. For example, acetone extract of Triphala, consisting equal parts of three medicinal plant fruits *Emblica officinalis*, *Terminalia belerica* and *Terminalia chebula*, were exhibited differential cytotoxic activity in several cancer cell lines including Shiongi 115, breast cancer MCF-7, prostate cancer PC3 and DU-145 cells (Kaur et al., 2005). Extracts from *A. catechu* bark and heartwood were exhibited variety of cytotoxicity on colon cancer COLO-205, cervix HeLa cancer cells and breast cancer MCF-7 (Nadumane and Nair, 2011; Ghate et al., 2014). The inhibitory activity of *M. oleifera* extract on human colon carcinoma (HCT-8, HCT-15, SW48, and SW480), lung cancer (A459) and pancreatic cancer cells (Panc-1, p34, and COLO 357) was recorded (Pamok et al., 2012; Berkovich et al., 2013; Tiloke et al., 2013).

Phytochemical studies of the tested plants have identified variety of bioactive compounds, including carotenoids, vitamins, minerals, tannins, gallic acid, ellagic acid, glycosides, alkaloids, sterols, 4-hydroxybenzoic acid, γ-sitosterol, quercetin, 3, 4', 7-trihydroxyl-3', 5-dimethoxyflavone, catechin, moringine, moringinine, phenol, and flavonoids, all of which possess strong antioxidant and anticancer activities (Govindarajan et al,., 2004; Baliga, 2010; Li et al., 2010; Krishnamurthy et al., 2015). These compounds may exert their antitumor activities by different mechanisms including free radical sequestration, electron donation, metal ion chelating, and gene expression regulation (Aherne and O'Brien, 2002; Ravishankar et al., 2013).

To further elucidate the mechanism by which these extracts induced growth inhibition, we evaluated whether these extracts can affect cell cycle progression in leukemia HL-60 and K562 cells. Flow cytometric DNA content analysis indicated that the selected extracts blocked cell cycle progression at G_0/G_1 phase. These findings are consistent with earlier studies reported that *Acacia* honey induced cytotoxicity on human lung cancer NCI-H460 and human A375 and murine B16-F1 melanoma cell lines by blocking cell cycle progression in G_0/G_1 phase and downregulation of Bcl-2 and P5 genes (Pichichero et al., 2010; Aliyu et al., 2013).

Further cytometric analysis exhibited that *A. catechu* at high concentration (100μg/mL) prevented K-562 cells from entering the G2/M phase due to increase the accumulation of the cells in this phase. Indeed, G2/M arrest is typically associated with DNA damage and affords the cell time to repair before proceeding with mitosis, thereby preventing the persistence of genomic mutations (Stark and Taylor, 2004). Similar results obtained by Sundarraj et al. (2012) who reported that *Acacia nilotica* extract and γ-Sitosterol were inhibited the cell proliferation in human breast MCF-7 and lung A549 cancer cells by blocking G2/M phase. It is noteworthy that, the absence of G2/M accumulation reported in the present study implied that the damage induced by these extracts are minimal or absent at least in the experimental condition.

The appearance of the characteristic sub-G_0/G_1 (sub-diploid) peak on a DNA histogram is a specific marker of apoptosis (Morgan, 2006). The strongest accumulation of sub-diploid peak was observed in HL-60 cells treated with 100 μg/mL of *T. bellerica* (18.8-fold higher compared to the control) and in K562 cells treated with 100 μg/mL of A. lattifolia (5.8-fold higher compared to the control). These data are consistent with findings of Ghate et al (2014) who demonstrated that flow cytometric analysis of 70% methanol extract of *T. bellerica* at high concentration (100 μg/mL) induced inducing apoptosis in lung A549 and breast MCF-7 cancer cell lines.

Interestingly, the absence of apoptosis in K562 cells by *A. Latifolia*, *T. bellerica* and *M. oleifera* at low or high concentrations within this experimental duration suggested that the examined extracts inhibited proliferation of K562 cells in cytostatic manner without killing tumor cells. According to Rixe and Fojo (2007), it is possible to distinguish between the cytostatic and the cytotoxic effect. Cytotoxic agents, at both high and low concentrations delay cell progression in both S and G2/M phase, but lethality occurs only in S-phase. By contrast, a cytotstatic agent delays cell progression in G1 phase, without lethality at intermediate drug concentrations. The cytostatic activity of whole plant extract on cancer cells is often much better than effect of their isolated active biological compounds, 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 (Smit et al., 1995; Katiyar et al., 2012).

To confirm whether the sub-G1 peak is due to apoptotic HL-60 cells, the DNA was examined by DNA gel-electrophoresis. To date, two major mechanisms of eukaryotic cell death have been identified: necrosis and apoptosis. Necrosis is characterized by random DNA fragmentation resulting in a smear on agarose gel. Apoptosis is characterized by cleavage of chromosomal DNA into oligonucleosomal DNA which detected upon agarose gel electrophoresis as distinct DNA laddering (Kim et al., 2005). In our experiments, incubation HL-60 with the extracts produced a smear pattern of DNA degradation upon agarose gel. A possible mechanism for these extracts was suggested to be necrosis rather than apoptosis, because of the appearance of sub-G1 peak and the non ladder type degradation of nuclear DNA in the early stage of cell death (Qian et al., 1995). This implied that, these extracts exerted their cytostatic activity on HL-60 cells by accumulation of cells in G1 phase of the cell cycle and reduction of cells in S and G2/M. Therefore, plant extracts could be proposed as adjuvant in cancer chemotherapy. Based upon the initial screening work reported here, further experiments are required to provide a better understanding of their molecular anticancer mechanisms involved in both *in vitro* and *in vivo* system.,

Acknowledgements

We are thankful to the Department of Science and Technology, Ministry of Science and Technology, India, for providing a postdoctoral grant under programme of C

V Raman International Fellowship for African Researchers to Kawthar A.E. Diab to work at Indian Institute of Integrative Medicine, Council of Scientific and Industrial Research, CSIR, Jammu.

References

Aherne SA, O'Brien NM (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition*, **18**, 75-1.

Aliyu M, Odunola OA, Farooq AD, et al (2013). Molecular mechanism of antiproliferation potential of *Acacia* honey on NCI-H460 cell line. *Nutr Cancer*, **65**, 296-4.

Baliga MS (2010). Triphala, Ayurvedic formulation for treating and preventing cancer: a review. *J Altern Complement Med*, **16**, 1301-8.

Berkovich L, Earon G, Ron I, et al (2013). *Moringa oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *BMC Complement Altern Med*, **13**, 212.

Biswas KS, Chowdhury A, Das J, et al (2012). Pharmacological potentials of *Moringa oleifera* lam. A review. *Inter J Pharma Sci Res*, **3**, 305-10.

Boik J (2001). Natural compounds in cancer therapy: promising nontoxic antitumor agents from plants & other natural sources. 1st edition, Minnesota, USA: Oregon Medical Press, pp 275-8.

Brusotti G, Cesari I, Dentamaro A, et al (2014). Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethnopharmacological approach. *J Pharm Biomed Anal*, **87**, 218-8.

Caceres AA, Saravia A, Rizzo S, et al (1992). Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, antiinflammatory and diuretic activity. *J Ethnopharmacol*, **36**, 233-6.

Chanda D, Bhushan S, Guru SK, et al (2012). Anticancer activity, toxicity and pharmacokinetic profile of an indanone derivative. *Eur J Pharm Sci*, **47**, 988-5.

Chitale VS, Behera MD, Roy PS (2014). Future of endemic flora of biodiversity hotspots in India. *PLoS One*, **9**, 115264.

Ghate NB, Hazra B, Sarkar R, et al (2014). Heartwood extract of *Acacia catechu* induces apoptosis in human breast carcinoma by altering bax/bcl-2 ratio. *Pharmacogn Mag*, **10**, 27-3.

Ghate NB, Hazra B, Sarkar R, et al (2014). Alteration of Bax/Bcl-2 ratio contributes to *Terminalia belerica*-induced apoptosis in human lung and breast carcinoma. *In vitro Cell Dev Biol Anim*, **50**, 527-7.

Govindarajan R, Vijayakumar M, Rao CV, et al (2004). Antioxidant potential of *Anogeissus latifolia*. *Biol Pharm Bull*, **27**, 1266-9.

Katiyar C, Gupta A, Kanjilal S, et al (2012). Drug discovery from plant sources: An integrated approach Ayu. 33, 10-9.

Kaur S, Michael H, Arora S, et al (2005). The *in vitro* cytotoxic and apoptotic activity of Triphala-an Indian herbal drug. *J Ethnopharmacol*, **97**, 15-20.

Kim R, Emi M, Tanabe K (2005). Caspase-dependent and -inde¬pendent cell death pathways after DNA damage. *Oncol Rep*, **14**, 595-9.

Krishnamurthy PT, Vardarajalu A, Wadhwani A, et al (2015). Identification and characterization of a potent anticancer fraction from the leaf extracts of *Moringa oleifera* L. *Indian J Exp Biol*, **53**, 98-3.

Li X, Wang H, Liu C, et al (2010). Chemical constituents of *Acacia* catechu. *China J Chin Mater Med*, **35**, 1425-7.

Morgan D (2006). The cell cycle: principles of control (primers in biology series). London, UK: New Science Press Ltd, pp 297.

Nadumane VK, Nair S (2011). Evaluation of the anticancer and cytotoxic potentials of *Acacia catechu* extracts *in vitro*. *J Nat Pharm*, **2**, 190-5.

Pamok S, Saenphet S, Vinitketkumnuen U, et al (2012). Antiproliferative effect of *Moringa oleifera* lam. and pseuderanthemum palatiferum (Nees) Radlk extracts on the colon cancer cells. *J Med Plants Res*, **6**, 139-5.

Patil UH, Gaikwad DK (2011). Ethno-pharmacological review of a herbal drug: *Anogeissus latifolia*. *Inter J Pharma Sci Res*, **2**, 41-3.

Pichichero E, Cicconi R, Mattei M, et al (2010). *Acacia* honey and chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. *Int J Oncol*, **37**, 973-81.

Qian L, Murakami T, Kimura Y, et al (1995). Saikosaponin A-induced cell death of a human hepatoma cell line (HuH-7): the significance of the 'sub-G1 peak' in a DNA histogram. *Pathol Int*, **45**, 207-14.

Rashed K, Potocnjak I, Giacometti J, et al (2014). *Terminalia bellerica* aerial parts ethyl acetate extract exhibits antioxidant, anti-inflammatory and antifibrotic activity in carbon tetrachloride-intoxicated mice. *J Fun Foods*, **8**, 319-30.

Ravishankar D, Rajora AK, Greco F, et al (2013). Flavonoids as prospective compounds for anti-cancer therapy. *Int J Biochem Cell Biol*, **45**, 2821-31.

Rixe O, Fojo T (2007). Is cell death a critical end point for anticancer therapies or is cytostasis sufficient? *Clin Cancer Res*, **13**, 7280-7.

Saxena A, Saxena AK, Singh J, et al (2010). Natural antioxidants synergistically enhance the anticancer potential of AP9-cd, a novel lignan composition from Cedrus deodara in human leukemia HL-60 cells. *Chem Biol Interact*, **188**, 580-90.

Smit HF, Woerdenbag HJ, Singh RH, et al (1995). Ayurvedic herbal drugs with possible cytostatic activity. *J Ethnopharmacol*, **47**, 75-4.

Stark GR, Taylor WR (2004). Analyzing the G2/M checkpoint. *Methods Mol Biol*, **280**, 51-82.

Stohs SJ, Bagchi D (2015). Antioxidant, Anti-inflammatory, and chemoprotective properties of *Acacia catechu* heartwood extracts. *Phytother Res*, [Epub ahead of print].

Sundarraj S, Thangam R, Sreevani V (2012). γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. *J Ethnopharmacol*, **141**, 803-9.

Tiloke C, Phulukdaree A, Chuturgoon AA (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC Complement Altern Med*, **13**, 226.

Vega-Avila E, Pugsley MK (2011). An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. *Proc West Pharmacol Soc*, **54**, 10-4.

Vichai V, Kirtikara K (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*, **1**, 1112-6.