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Research Article

SCREENING OF BIOPROTECTIVE PROPERTIES OF VARIOUS PLANT EXTRACTS AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING OF *ADENANTHERA PAVONINA* STEM EXTRACT

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ABSTRACT

Objective: The search for various phytotherapeutic compounds is on rise due to a complex multifactorial phenomenon called drug resistance. The present study investigates the cytotoxic, antioxidant, and antiproliferative potential of methanolic extracts of *Clitorea ternatea, Averrhoa bilimbi, Phyllanthus acidus, Tecoma stans, Curcuma aromatica, Anethum graveolens, Adhatoda vasica, Markhamia lutea, Spathodea companulata,* and *Adenanthera pavonina*.

Methods: The plant parts were extracted with methanol and screened for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging abilities. The cytotoxic activity of the extracts was investigated on HeLa and HCT116 cells through 3-(4,5-dimethylthiazol2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and cell cycle was analyzed by flow cytometry to determine the antiproliferative activity of the extracts. The stem extract of *A. pavonina* was further subjected to gas chromatography-mass spectrometry (GC-MS) analysis for purification of the compounds of interest. A two-way ANOVA was done to estimate the effect of the extract between samples remembered at $p<0.05$ level.

Results: Among all the studied samples, the extract of *A. pavonina* (stem) showed significant scavenging activity of 70.23% and 76.32% of scavenging compared to 74.58 % and 81.13% of that of reference standard in ABTS and DPPH assay, respectively. GC-MS analysis of the extract revealed the presence of 17 phytocompounds. MTT assay revealed that this extract (SB19) had promising cytotoxic activity against the two cancer cell lines, HCT116 and HeLa with inhibitory concentration 50% IC_{50} values of 25.86±0.21 µg/ml and 39.89±0.11 µg/ml, respectively. The extract treatment caused significant arrest in G2M phase of cell cycle.

Conclusion: *A. pavonina* (stem) extract displayed significant antioxidant and antiproliferative activity and can be considered as a potential candidate drug for anticancer studies.

Keywords: Antioxidants, 3-(4,5-dimethylthiazol2yl)-2,5-diphenyl tetrazolium bromide assay, Antiproliferative, Gas chromatography-mass spectrometry, *Adenanthera pavonina.*

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INTRODUCTION

Plants are natural source of novel therapeutics as they contain various phytoconstituents which serve as a source of bioactive compounds with significant pharmacological action. These phytochemicals are referred to as secondary metabolites which are formed during the plants' normal metabolic processes [1] and include alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes, and terpenoids [2]. Oxidative stress is caused by free radicals which are produced in our body due to aerobic respiration and substrate oxidation [3-7]. When these free radicals are present in excess, they exert oxidative damage to cellular biomolecules such as membrane lipids, cellular proteins, DNA, and enzymes, eventually leading to many chronic diseases. However, the antioxidant actions of endogenous enzymes as well as natural and synthetic antioxidants can balance the production of free radicals [8,9]. Mode of action of antioxidants includes several mechanisms such as prevention of chain initiation, decomposition of peroxidases, radical scavenging, chelating of transition metal ion catalysts, and prevention of continued hydrogen abstraction [10].

Research suggests that phytochemicals found in botanicals may help in combating various diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, and urinary tract infections and in slowing the aging process. Being rich source of antioxidants, consumption of several plants was recommended [11,12]. Cancer is the second biggest killer after heart disease in India and the data from the WHO World Cancer Report released in 2015 indicate that in India, there are 7 lakh new cancer cases per year, killing over 3.5 lakh people every year. Certain preclinical studies provide evidence that phytochemicals can prevent colorectal cancer and other cancers due to their polyphenol antioxidant and anti-inflammatory effects [13-15].

The present work was thus planned with the aim to explore the bioactivities of these plant extracts by assessing their cytotoxic, antiproliferative, and antioxidant efficacy. Based on earlier investigation carried out for evaluation of their antifungal activity on *Candida glabrata* and screening of phytoconstituents and the current assessment, the active stem extract of *Adenanthera pavonina* was further subjected to gas chromatography-mass spectrometry (GC-MS) analysis for identification of the components present in the extract.

METHODS

Plant material

Fresh plant parts were collected during January to June 2013 from various regions in Bengaluru, Karnataka, India. Identification and authentication of the plants were done by Dr. Ramakrishna T M,

Department of Biological and Life Science, Bangalore University, and documented with their characteristic features. Plant material was washed, shade-dried and then homogenized to fine powder, and stored in airtight bottles with proper labeling for future use. The labeling provided for various plant parts were as follows: SB1 (*Clitorea ternatea* leaf), SB2 (*C. ternatea* flower), SB3 (*Averrhoa bilimbi* fruit), SB4 (*A. bilimbi* leaf), SB5 (*Phyllanthus acidus* leaf), SB6 (*P. acidus* fruit), SB7 (*Tecoma stans* flower), SB8 (*T. stans* leaf), SB9 (*Curcuma aromatica* leaf), SB10 (*C. aromatica* rhizome), SB11 (*Anethum graveolens* leaf), SB12 (*A. graveolens* stem), SB13 (*Adhatoda vasica* leaf), SB14 (*A. vasica* flower), SB15 (*Markhamia lutea* leaf), SB16 (*M. lutea* flower), SB17 (*Spathodea companulata* leaf), SB18 (*S. companulata* flower), SB19 (*Adenanthera pavonina stem*), and SB20 (*A. pavonina* leaf).

The chemicals methanol, ethanol, dimethyl sulfoxide (DMSO), ammonium per sulfate, triton were procured from qualigens; 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, colchicine, and propidium iodide (PI) were purchased from Sigma-Aldrich, USA. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution, 3-(4,5-dimethylthiazol2yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent, and RNaseA were procured from Hi-Media, Bengaluru.

Extraction of plant material

Crude plant extracts were prepared by Soxhlet extraction method, wherein the powdered plant materials were extracted with methanol. The extracts were then taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their future use.

ABTS assay

The assay is performed as per the study by Auddy *et al*., 2003 [16]. ABTS 7 mM (38.4 mg in 10 ml) and ammonium persulfate 2.45 mM (5.59 mg in 10 ml) were prepared in phosphate buffer saline (PBS, pH 7.4). ABTS radical cations are produced by reacting ABTS (7 mM) and ammonium persulfate (2.45 mM) and incubating the mixture at room temperature in dark for 16 hrs. The solution thus obtained is further diluted with PBS to give an absorbance of 1.000. Different concentrations of the methanolic plant extracts and the reference standard quercetin (1 mg in 10 ml PBS) are added to 950 µml of ABTS working solution to give a final volume of 1 ml, made up by adding PBS. The absorbance is recorded immediately at 734 nm. The percent inhibition is calculated at different concentrations and the inhibitory concentration 50% (IC_{50}) values are calculated by Log-Probit analysis. The inhibition was calculated in following way: I (%) = 100 × $(A_0-A_1)/A_0$; where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. A percent inhibition versus concentration curve was plotted, and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. The lower the IC_{50} value indicates high antioxidant capacity.

DPPH assay

Screenings of antioxidant activity of the extracts were carried out by DPPH free radical scavenging assay [17,18] using ultraviolet spectrophotometric methods. According to the protocol, 200 µL of test solutions from different extracts was dissolved in methanol. This solution was then combined with 1.8 ml of DPPH methanol solution. After being mixed, solutions were kept at room temperature, in the dark for 30 minutes incubation. After the reaction, the absorbance was recorded at 517 nm. Methanol was used as a blank, DPPH solution was used as negative control (A_0), and quercetin (20 mg/ml) was used as positive control standard. The antioxidant activity was given in terms of IC_{50} value. All the experiments were carried out in triplicates. The % scavenging effect was obtained from the formula: scavenging effect (%) = $(A_0-A_1)/A_0$ × 100, where A_0 was the absorbance of the control reaction and A_1 was the absorbance of the sample of the tested extracts. Percentage of inhibition was calculated using the following formula: % inhibition = $[(A_{negative}-A_{test})/A_{negative}]$ × 100 (A is absorbance).

Cytotoxicity assay

Cytotoxic activity of methanolic plant extracts against two cancer cell lines was evaluated by MTT assay. HeLa and HCT-116 cell lines were cultured and grown in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 2% antibiotic and maintained at 37°C in 5% CO_2 for 24 hrs in a humid environment. 50,000 cells of HeLa and HCT-116 cell lines were plated in triplicates in 96-well plates with DMEM and incubated for 24 hrs at 37°C. Varying concentration of plant extracts was taken in FBS-free DMEM and incubated for 24 hrs. 100 µl of 5 mg/10 ml MTT solution in PBS was added to each well and incubated for 2-3 hrs. After incubation, MTT reagent was discarded. 100 µl of DMSO was added to each well that will dissolve the formazan crystals. The plates were read at 590 nm using a microplate reader. Percent viability is calculated using the formula: % cell viability = (OD control−OD sample)/OD control × 100.

Cell cycle analysis

1×10^6 cells from HCT-116 and HeLa cell cultures were seeded in 6-well plates containing 2 ml of complete DMEM, were plated in two different independent experimental setups, and were grown for 24 hrs in 5% CO_2 incubator at 37°C. After 24 hrs of incubation, cells are treated with or without 200 and 320 µg/ml *A. pavonina* stem extract. 20 µM colchicine was used as positive control and 1% DMSO as negative control in 1 ml/well of DMEM and was incubated for 24 hrs. Thereafter, cells were collected and pelleted at 1500 rpm for 5 minutes at room temperature and the supernatant was discarded. The pellet was resuspended gently in 1× PBS and was fixed overnight at 4°C in 2 ml of fixing solution (20% PBS in 70% ethanol). The suspension was centrifuged at 4000 rpm for 10 minutes at room temperature and the supernatant was discarded. Cells were washed twice with ice-cold 1× PBS. Later, cells were incubated for 15 minutes or 1 hr at room temperature in 500 µl of PI solution containing 0.05 mg/ml PI and 0.05 mg/ml RNase A in PBS. The percentage of cells in various stages of cell cycle in compounds treated and untreated populations was determined using FACSCalibur (BD Biosciences, San Jose, CA).

GC-MS analysis

A GC-MS analysis was performed to study the phytochemical components present in the methanol extract of stem of *A. pavonina*. GC-MS analysis was carried out on a GC Clarus 500 PerkinElmer system comprising a GC-MS instrument employing the following conditions: GC-MS analysis was carried out on a column Restek RtxR – 5, (30 meter X 0.25 mm 5% diphenyl / 95% dimethyl polysiloxane), operating in electron impact mode at 70eV. Injection temperature was maintained at 280°C, helium flow rate as 1 ml/min and ion source temperature at 230°C. Injection was performed in the splitless mode and the volume was 1 µl. The column oven temperature was programmed as 40-280°C at a rate of 6°C/min injection mode, wherein the instrument was set to an initial temperature of 40°C and was maintained at this temperature for 5 minutes. At the end of this period, the oven temperature was raised up to 280°C which was maintained for 15 minutes. The mass spectra of compounds in samples were obtained by electron ionization at 70 eV and in the mass range of 50-700 mass units. Total GC-MS run time is 60 minutes. The essential chemical constituents were identified by interpreting the on mass spectra of GC-MS using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight, and structure of the compounds of methanol extract of stem of *A. pavonina* were ascertained.

Statistical analysis

All of the statistical calculations employed in the present context were carried out with the SPSS10.0. Results were expressed as the mean±standard error of triplicates. A two-way ANOVA was used for statistical analysis; $p>0.05$ was considered statistically significant.

RESULTS

ABTS assay

The methanol extract of the stems of *A. pavonina* was fast and effective scavenger of the ABTS radical (Fig. 2) with an IC_{50} value

of 19.67±0.21 µg/ml compared to 5.68 µg/ml of quercetin and the increase in this activity was dose dependent. Methanolic extracts of flower of *A. vasica* and *C. aromatica* rhizome also exhibited prominent ABTS quenching activity with an IC_{50} value of 28.23±0.11 and 29.57±0.22 µg/ml, respectively. Other plant extracts which exhibited ABTS scavenging activity includes *P. acidus* leaf, *T. stans* leaf, *A. vasica* leaf, *S. companulata* leaf, and *M. lutea* flower with IC_{50} values 36.61±0.05, 37.71±0.11, 40.23±0.22, 41.25±0.12, and 53.2±0.10 µg/ml, respectively. The extracts of *A. pavonina* stem, *P. acidus* leaf, *A. vasica* flower, *C. aromatica* rhizome, *T. stans* leaf, *S. companulata* leaf, *A. vasica* leaf, and *M. lutea* exhibited percentage inhibition of 70.23%, 64.13%, 63.93%, 58.58%, 57.05%, 56.49%, 51.24%, and 33.51%, respectively, compared to 74.58% of quercetin at 100 µg/ml concentration (Fig. 1). A two-way ANOVA between the concentration and different samples was conducted to compare the effect of samples on ABTS scavenging activity. There was a significant effect of different samples remembered at p<0.05 level. The significance effect on the ABTS scavenging activity between the samples and varying concentrations: F(6, 48)=147.3328, p=3.56E-29 and F(8,48)=4.386676, p=0.00497.

DPPH assay

Among the plants extracts as seen in DPPH assay, *A. pavonina* stem extract exhibited highest antioxidant activity with IC_{50} value of 25.01±0.25 µg/ml compared to 4.411 µg/ml of the standard quercetin. Other plant extracts which exhibited antioxidant activity were *P. acidus* leaf, *T. stans* leaf, *M. lutea* flower, *S. companulata* leaf with IC_{50} value of 43.23±0.31, 43.6±0.11, 53.92±0.44, and 35.67±0.20 µg/ml, respectively. The scavenging activity was presented as the percentage of inhibition of DPPH free radicals (Fig. 4), wherein at 100 µg/ml maximum inhibitory effect of 76.32% was shown by *A. pavonina* stem extract compared to 81.13% of reference standard. Other extracts of *P. acidus* leaf, *T. stans* leaf, *M. lutea* flower, *S. companulata* leaf exhibited 47.89%, 59.87%, 51.58%, and 55.89% of inhibition, respectively (Fig. 3). A two-way ANOVA between the concentration and different samples was conducted to compare the effect of samples on DPPH scavenging activity. There was a significant effect of different samples remembered at p<0.05 level. The significance effect on the DPPH scavenging activity between the samples and varying concentrations, F(6, 36)=60.584, p=2.44E-17 and F(6,36)=8.512926, p=0.00517.

MTT assay

Among the various plant extracts used for the assay extracts of *A. bilimbi* leaf, *P. acidus* leaf, *A. vasica* leaf, *M. lutea* leaf, and *A. pavonina* stem demonstrated cytotoxic effect on HeLa cells (Fig. 7) with IC_{50} values 72.35±0.23, 57.83±0.13, 91.23±0.31, 81.12±0.33, and 39.89±0.11 µg/ml, respectively. These same plant extract samples have shown dose-dependent manner of cytotoxicity in HCT116 cells as well (Fig. 6). The IC_{50} value of *A. bilimbi* leaf, *P. acidus* leaf, *A. vasica* leaf, *M. lutea* leaf, and *A. pavonina* stem extract was 87.34±0.41, 45.67±0.05, 85.72±0.31, 74.53±0.13, and 25.86±0.21 µg/ml, respectively (Fig. 7).

Cell cycle analysis

The effect of *A. pavonina* stem extract at two different concentrations on cell cycle in HeLa cells and HCT116 cell lines as analyzed by flow cytometry is depicted in Fig. 8. The *A. pavonina* stem extract treatment on HeLa cells and HCT116 cells has significantly arrested G2M phase of cell cycle at 23.94% and 15.56%, respectively, at a concentration of 200 µg/ml and 29.86% and 25.37%, respectively, at a concentration of 320 µg/ml compared to untreated cells with 18.93% and 14.45% arrest, respectively. Colchicine has exhibited a cell cycle arrest of HeLa cells and HCT116 cells at 48.38% and 50.33%, respectively, in G2M phase (Fig. 9).

GC-MS analysis

The test sample *A. pavonina* stem extract was subjected to GC-MS and the total separated peaks are shown in Fig. 10. The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the sample. All 17 compounds were identified from the GC-MS analysis of the sample SB19 extract exhibiting various phytochemical activities

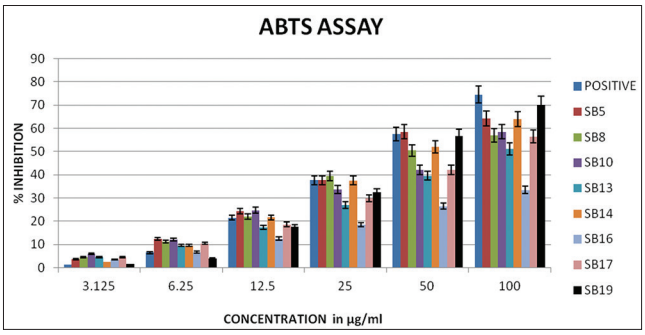

Fig. 1: The percentage inhibition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay. All the values were average of triplicates. Quercetin is used as positive control

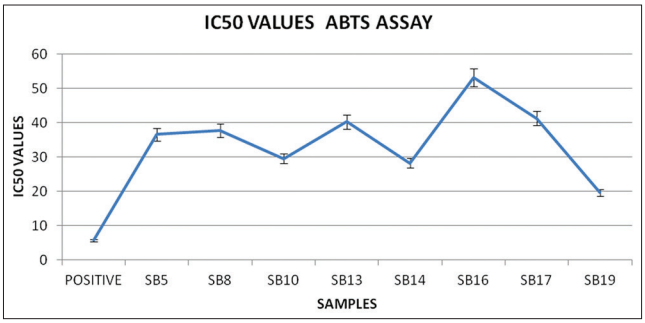

Fig. 2: The inhibitory concentration 50% values of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay. All the values were average of triplicates

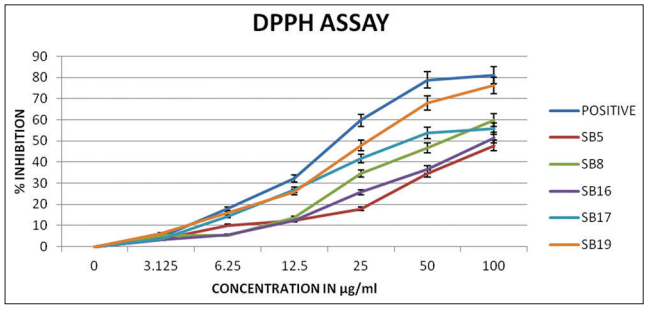

Fig. 3: The percent inhibition of 1,1-diphenyl-2-picrylhydrazyl assay. All the values were average of triplicates. Quercetin is used as positive control

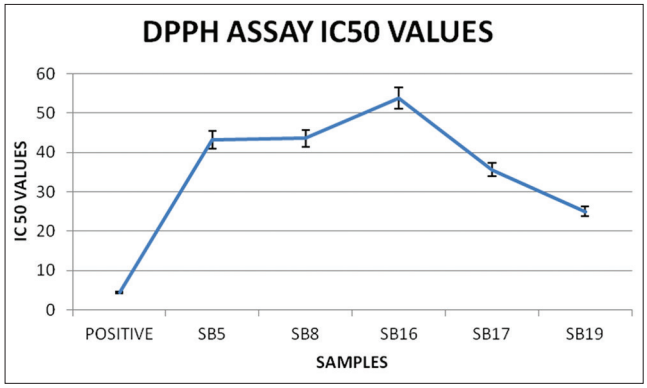

Fig. 4: The inhibitory concentration 50% values of 1,1-diphenyl-2-picrylhydrazyl assay. All the values were average of triplicates

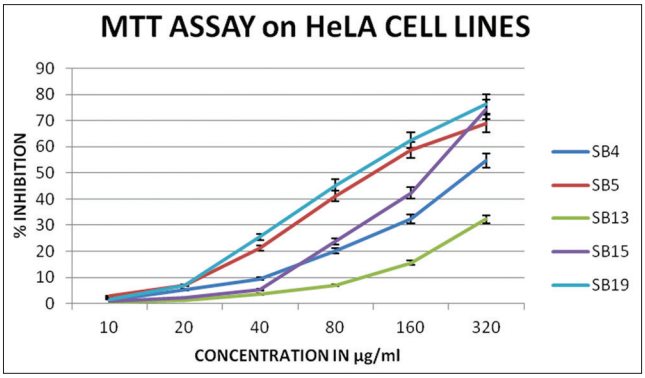

Fig. 5: The 3-(4, 5-dimethylthiazol2yl)-2, 5-diphenyl tetrazolium bromide cytotoxicity values of the extracts on HeLa cell lines. All the values were average of triplicates % ± standard error

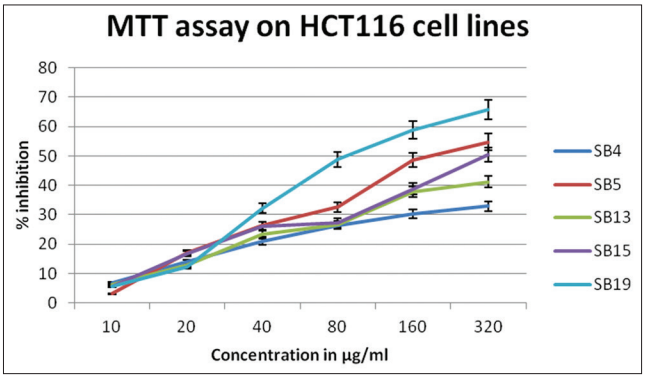

Fig. 6: The percent inhibition values of the extracts obtained in 3-(4,5-dimethylthiazol2yl)-2,5-diphenyl tetrazolium bromide assay on HCT116 cell lines. All the values were average of triplicates % ± standard error

and were predominantly responsible for the antifungal activity found in the extract against the pathogenic species mentioned. The retention time and percentage peak of various bioactive compounds are presented in Table 1. The major phytoconstituents present in the stem extracts of *A. pavonina* were isobutyl nitrate, 3,4 hexane dione, oxalic acid butyl propyl ester, isonitropropane, oxalic acid, ally pentyl ester, 2-benzyl-1,3-dioxolane, 1,3-dioxolane, 2-benzyl-1,3-dioxolane, cyclopentasiloxane, [(2,4,4,6,6,8,8-heptamethylcyclotetrasiloxan-2-yl)oxy]nonamethyl-, 2-heptyl-1,3-dioxolane, 3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris tetrasiloxane, malonic acid, bis(2-trimethylsilylethyl ester, acetic acid, [o-(trimethylsiloxy) pentyl]-, trimethylsilyl ester, methyl-alpha.-d-ribofuranoside, 3-methylmannoside, 2,3,4,5-tetrahydroxypentanal, and methyl 4-o-methyl-d-arabinopyranoside.

DISCUSSION

In recent years, various research validations have accentuated the importance of antioxidants in prevention and treatment of diseases by reducing the deleterious effects of free radicals [19]. Free radicals are one of the major factors responsible for inducing DNA mutation through various oxidative processes, resulting in the initiation of carcinogenesis [20]. The antioxidants through their ability to quench the free radicals and reactive oxygen bring about regression of premalignant lesions and inhibit their development into cancer [21]. Furthermore, the endogenous antioxidant system responsible for preventing the formation of free radicals in the body can be improved by supplementing natural sources with antioxidant property [22]. Botanicals being a rich source of antioxidants act as efficient free radical scavengers and play significant role in chemoprevention.

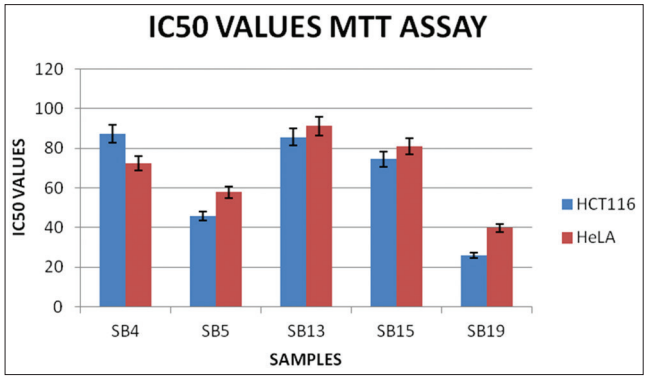

Fig. 7: The inhibitory concentration 50% values of the extracts obtained in 3-(4,5-dimethylthiazol2yl)-2,5-diphenyl tetrazolium bromide assay on both the cell lines, HCT116 and HeLa. All the values were average of triplicates

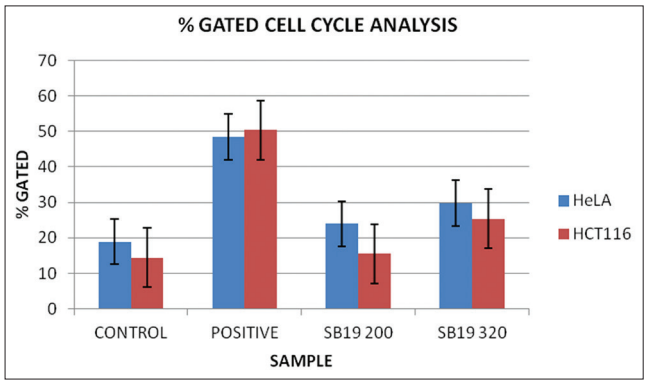

Fig. 8: The percent cell viability values at G2M phase. All the values were average of triplicates. The values were expressed as % ± standard error. The cell viability assay was done on both HeLa and HCT116 cell lines

In the present investigation, the result of ABTS assay indicated that among the eight plant extracts which demonstrated antioxidant activity *A. pavonina* stem exhibited most potent ABTS scavenging activity. Based on the percentage inhibition, the plant extracts can be ranked in the following descending order: *A. pavonina* stem > *P. acidus* leaf > *A. vasica* flower > *C. aromatica* rhizome > *T. stans* leaf > *S. companulata* leaf > *A. vasica* leaf > *M. lutea* flower. The DPPH assay results revealed that *A. pavonina* stem extract exhibited most promising antioxidant activity followed by extracts of *S. companulata* leaf, *P. acidus* leaf, *T. stans* leaf, and *M. lutea* flower.

Studies on these plants by other researchers also indicate their radical scavenging activity. Strong antioxidant activity is shown by aqueous extract of the decoction prepared with barks of *A. pavonina* and *Thespesia populnea* with an effective concentration 50% (EC_{50}) value of 7.24±0.49 µg/ml [23], and also, it is demonstrated that the bark extract of *A. pavonina* has an EC_{50} value of 58.68 µg/ml [24]. The methanolic extract of fruit part of *P. acidus* exhibits moderate to good antioxidant activity in a dose-dependent manner and is found to contain flavonoid compounds [25]. Strong antioxidant properties were confirmed in the ethanol and methanol extract fractions of *T. stans*, wherein at a concentration of 0.1 mg/ml, the scavenging activity of ethanol and methanol extracts [26] reached 56.88% and 58.92%. Research reveals that the antioxidant mechanisms of *S. companulata* flower and bark extracts are distinct from each other and they present significant antioxidant capacity within a biological system in the presence of Fe^{3+} ascorbic acid [27]. Certain species of *Markhamia* such as the *Morchella tomentosa* methanolic extract indicate high DPPH radical scavenging capacity and antioxidant activity [28]. High concentration of antioxidant

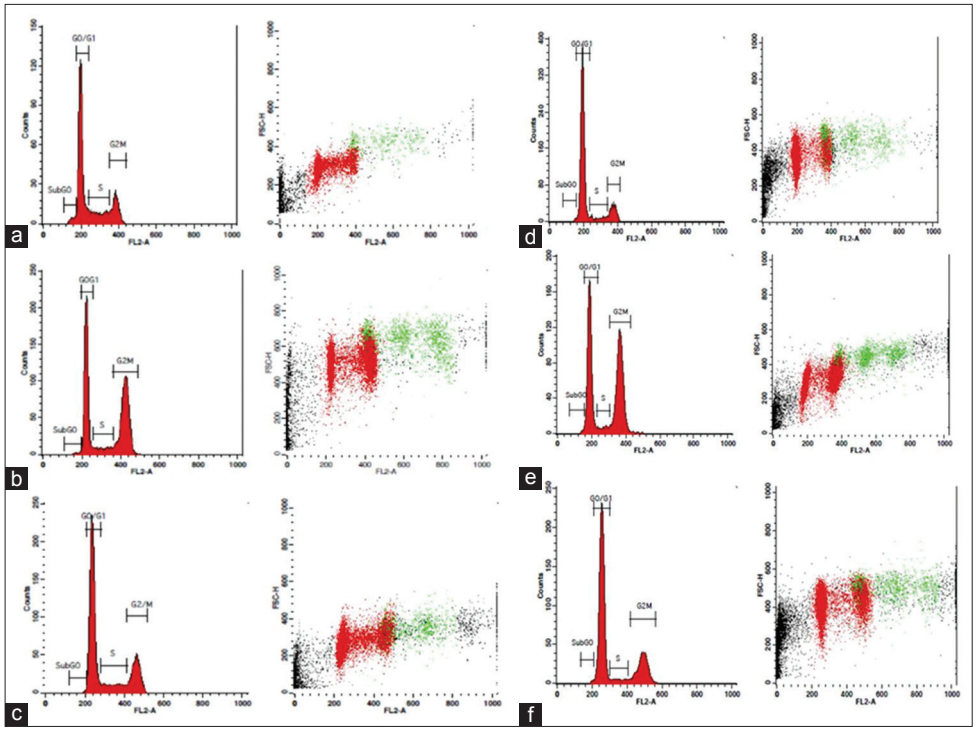

Fig. 9: Flow cytometry plots of the test sample *Adenanthera pavonina* stem extract against HeLa and HCT116 cell lines. (a) HeLa cells treated with 1% dimethyl sulfoxide (DMSO) as control; (b) HeLa cells treated with 20 µM of colchicine; (c) HeLa cells treated with 200 µg/ml of test sample extract; (d) HCT116 cells treated with 1% DMSO as control; (e) HCT-116 cells treated with 20 µM of colchicine; (f) HCT116 cells treated with 320 µg/ml of test sample extract

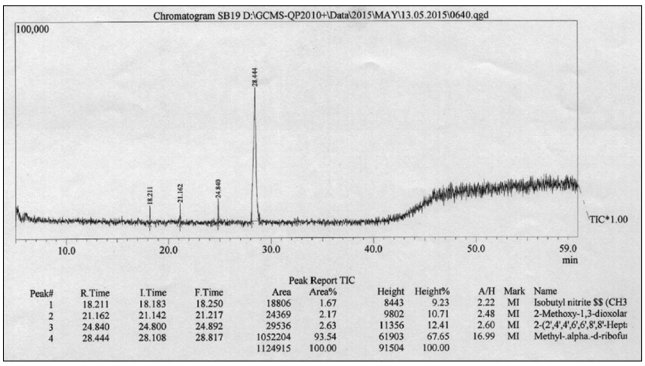

Fig. 10: Gas chromatography-mass spectrometry chromatograms of methanolic extract from the stem of *Adenanthera pavonina* (SB19)

phytochemicals such as polyphenolic compounds and flavonoids are present in *A. vasica* and thus the plant shows strong total antioxidant activity [29].

Investigation on the cytotoxic efficacy of various plant extracts on HeLa and HCT116 cell line by utilizing high-throughput MTT assay demonstrated that five of the plant extracts have cytotoxic effect on both HeLa and HCT116 cell line in a dose-dependent manner. *A. pavonina* stem extract exhibited potent cytotoxic efficacy in both the cell lines. In HeLa cell lines, the extracts of *A. pavonina* stem, *M. lutea* leaf, *P. acidus* leaf, *A. bilimbi* leaf, and *A. vasica* leaf exhibited 76.34%, 74.35%, 68.91%, 54.67%, and 32.23% of cytotoxicity at 320 µg/ml, respectively (Fig. 5). Most promising cytotoxicity in HeLa cell lines was thus shown by *A. pavonina* stem and *M. lutea* leaf extracts followed by the moderate activity of *A. bilimbi* leaf, *P. acidus* leaf extract and mild activity of *A. vasica* leaf extract. In HCT116 cell lines, the plant extracts of *A. pavonina* stem, *P. acidus* leaf, *M. lutea* leaf, *A. vasica* leaf, and *A. bilimbi* leaf demonstrated cytotoxicity of 65.78%, 54.77%, 50.43%, 41.23%, and 32.93% at 320 µg/ml, respectively. Among the five plants extracts, *A. pavonina* stem extract exhibited potent cytotoxic efficacy in both the cell lines. Assessment of cytotoxic potential of methanolic extract of *A. bilimbi* fruits using brine shrimp lethality bioassay suggests significant activity [30]. Studies reveal significant *in vitro* cytotoxic activity of ethyl acetate extract of leaves of *P. acidus* against Hep G2 and DLA cell lines [31]. *A. vasica* extract possesses promising anticancer activity [32]. Vasicine acetate obtained by acetylation of the alkaloid vasicine isolated from ethanolic extracts of leaves of *A. vasica* showed potent cytotoxic activity against A549 lung adenocarcinoma cancer cell with an IC_{50} value of 2000 µmg/mL. The decoction containing *A. pavonina* L. and *T. populnea* L bark extracts possesses potent antiproliferative and cytotoxic activities (Silva *et al.*, 2011).

Based on the results of antioxidant assay and cytotoxicity assay, *A. pavonina* stem extract was subjected to assessment of antiproliferative activity on HeLa and HCT116 cell lines by flow cytometry analysis. The investigation revealed that *A. pavonina* stem extract significantly arrests the cell cycle at G2M phase and induces apoptosis.

In addition, the present study aimed to isolate the phytoconstituents by GC-MS profiling of active plant extract. Thus, the following phytoconstituents were isolated from the stem extract of *A. pavonina*: Isobutyl nitrate, 3,4 hexane dione, oxalic acid butyl propyl ester, isonitropropane, oxalic acid, allypentyl ester, 2-benzyl-1,3-dioxolane, 1,3-dioxolane, 2-benzyl-1,3-dioxolane, cyclopentasiloxane, [(2,4,4,6,6,8,8-heptamethylcyclotetrasiloxan-2-yl)oxy]nonamethyl-, 2-heptyl-1,3-dioxolane, 3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris tetrasiloxane, malonic acid, bis(2-trimethylsilylethyl ester, acetic acid,[o-(trimethylsiloxy)pentyl]-, trimethylsilyl ester, methyl-alpha.-d-ribofuranoside, 3-methylmannoside, 2,3,4,5-tetrahydroxypentanal, and methyl 4-o-methyl-d-arabinopyranoside.

Among the various organic acids evaluated for their antifungal activity oxalic acid has exhibited a potent antifungal effect on the growth of four fungal species *Aspergillus flavus, Penicillium purpurogenum,*

Table 1: The phytochemical constituents obtained on GC chromatogram with NIST library match

S. No.	R_{index}	Compound name	Molecular formula	Molecular weight
GC chromatogram with NIST library match of peaks with RT 18.208 minutes				
1	544	Isobutyl nitrate	$C_4H_9NO_2$	103
2	1039	3,4 hexane dione	C9$H_{16}O_2$	156
3	1250	Oxalic acid butyl propyl ester	$C_9H_{16}O_4$	188
4	637	Isonitropropane	$C_3H_7NO_2$	8
5	1340	Oxalic acid, ally pentyl ester	$C_{10}H_{16}O_4$	200
GC chromatogram with NIST library match of peaks with RT 21.152 minutes				
6	715	2-methoxy-1,3-dioxolane	$C_4H_8O_3$	104
7	578	1,3-dioxolane	$C_3H_6O_2$	74
8	1312	2-benzyl-1,3-dioxolane	$C_{10}H_{12}O_2$	164
GC chromatogram with NIST library match of peaks with RT 24.842 minutes				
9	1716	Cyclopentasiloxane, [(2,4,4,6,6,8,8-heptamethylcyclotetrasiloxan-2-yl) oxy] nonamethyl-	$C_{16}H_{48}O_{10}Si_9$	652
10	1235	2-heptyl-1,3-dioxolane	$C_{10}H_{20}O_2$	172
11	1612	3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris tetrasiloxane	$C_{17}H_{50}O_7Si_7$	562
12	1468	Malonic acid, bis (2-trimethylsilylethyl ester	$C_{13}H_{28}O_4Si_2$	304
13	1566	Acetic acid,[o-(trimethylsiloxy) pentyl]-, trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$	296
GC chromatogram with NIST library match of peaks with RT 28.442 minutes				
14	1406	Methyl-alpha.-d-ribofuranoside	$C_6H_{12}O_5$	164
15	1714	3-methylmannoside	$C_7H_{14}O_6$	194
16	1436	2,3,4,5-tetrahydroxypentanal	$C_5H_{10}O_5$	150
17	1359	Methyl 4-o-methyl-d-arabinopyranoside	$C_7H_{14}O_5$	178

NIST: National Institute Standard and Technology, GC: Gas chromatography

Rhizopus nigricans, and *Fusarium oxysporum*, wherein the high acidity appeared for oxalic acid was 0.14 at a high concentration of 10% [33]. Oxalic acids may exhibit antioxidant properties by chelating Fe^{2+}. This ability of oxalic acid is of great importance as it reduces the availability of Fe^{2+} for oxidation by H_2O_2 to generate the highly reactive hydroxyl radicals. 1,3-dioxolanes exhibited significant antifungal activity against *Candida albicans* while most of the compounds have also shown significant antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis,* and *Pseudomonas aeruginosa* [34]. The antimycotic effect of six new polyazole derivatives from 2-(2,4-dichlorophenyl)-1,3-dioxolane was studied against pathogenic fungi for humans and animals. Remarkable effect was shown by these compounds on filamentous fungi *Aspergillus fumigatus* and *Scedosporium apiospermum* which were as effective as the two references ketoconazole and oxiconazole [35].

The apoptotic and cytotoxic effects of the alpha-diketone derivatives 2,3- and 3,4-hexanediones were investigated in three non-neuronal cell lines (MCF7, HepG (2), and CaCo-2) and in the neuroblastoma line, SH-SY5Y. 2,3- and 3,4-hexanediones showed apoptosis over the concentration range 1-1.6 mM, with 3,4-hexanedione being the more potent compared to the 2,3-isomer. These alpha-diketones may also have therapeutic implications as they display a degree of toxic selectivity toward neuroblastoma cells [36]. Studies were carried out to compare the cytotoxic potentiality of hexanedione food additives (2,3- and 3,4 isomers) with that of neurotoxic hexane metabolite 2,5-hexanedione in the human SK-N-SH neuroblastoma line. The IC_{50} value of 3,4-hexanedione (3.5±0.1 mM), indicated that this compound has approximately seven-fold greater toxic effect on cells compared with that of 2,5 derivative (IC_{50}=22.4±0.2 mM). Similarly, flow cytometry indicated that the 3,4-hexanedione derivative caused interruption in the neuroblastoma cell cycle and induced apoptosis up to 60.4±0.5% [37]. 2-benzyl-1,3-dioxolane exhibits a broad spectrum of biological activities such as antifungal [38], antibacterial [39,40], and antineoplastic [41] activities.

CONCLUSION

The search for such natural products has revolutionized the drug discovery program. The present study revealed the pharmacological potentials of various plants among which *A. pavonina* stem extract exhibited promising antioxidant, cytotoxic, and antiproliferative potential. To the best of our knowledge, this is the first report on the antiproliferative activity of *A. pavonina* stem extract on HeLa and HCT116 cell lines and its phytoconstituent characterization by GC-MS technique. Thus, these plants can be further studied for the development of new effective anticancer drugs.

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