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

SHUBHA BHADRAN1, SANGEETHA ANNIE GEORGE2, SUDHAKAR MALLA3, HARINI BP4

1Department of Genetics, Indian Academy Centre for Research & Post Graduate Studies, Bengaluru, Karnataka, India. 2Department of Zoology, Indian Academy Centre for Research & Post Graduate Studies, Bengaluru, Karnataka, India. 3Department of Biotechnology, Indian Academy Centre for Research & Post Graduate Studies, Bengaluru, Karnataka, India. 4Department of Zoology, Bangalore University, Bengaluru, Karnataka, India. Email: shubhabhadran@yahoo.co.in

Received: 25 February 2017, Revised and Accepted: 13 April 2017

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 peroxides, 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 activity. 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 species 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 (Clitoria ternatea leaf), SB2 (C. ternatea flower), SB3 (Averrhoa bilimbi), 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 campanulata leaf), SB18 (S. campanulata 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-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent, and RNase A were procured from Hi-Media, Bangalore.

**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 stirred to keep it in dark for 16 hrs. The solution thus obtained is further diluted with water until 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 per sulfate (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 µl 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 - A_{0})/A_{0}, where A_{0} is the absorbance of the control, A 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 same sample of the tested extracts. Percentage of inhibition was calculated using the following formula: % inhibition = [(A_{negative} - A_{sample})/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% CO₂, 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 PBS-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% CO₂, incubator at 37°C. After 24 hrs of incubation, cells were 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 Rtx-5 – 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 at 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 SPSS 0.0. 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. acida* 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 53.22±0.10 µg/ml, respectively. The extracts of *A. pavonina* stem, *P. acida* 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%, 81.13% of reference standard. Other extracts of flower, stem exhibited percentage inhibition of 76.32% was shown by *P. acida* 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. acida* leaf, *A. vasica* leaf, *M. lutea* leaf, and *A. pavonina* stem extract was 97.34±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.
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-trimethylsilyl) 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 compounds [22]. Botanicals being a rich source of antioxidants act as efficient free radical scavengers and play significant role in chemoprevention.

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<sub>50</sub>) value of 7.24±0.49 µg/ml [23], and also, it is demonstrated that the bark extract of *A. pavonina* has an EC<sub>50</sub> 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 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<sup>3+</sup> 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...
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 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 µg/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 *Adenanthera pavonina* (SB19):

- 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-3-ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(tetrasiloxane, malonic acid, bis(2-trimethylsilyl) 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,*
| S. No. | R<sub>index</sub> | Compound name | Molecular formula | Molecular weight |
|-------|------------------|----------------|-------------------|------------------|
| 1     | 544              | Isobutyl nitrate | C<sub>9</sub>H<sub>10</sub>N | 153              |
| 2     | 1039             | 3,4-hexane dione | C<sub>6</sub>H<sub>10</sub>O | 124              |
| 3     | 1250             | Oxalic acid butyl propyl ester | C<sub>9</sub>H<sub>12</sub>O<sub>4</sub> | 188              |
| 4     | 637              | Isonitropropane | C<sub>5</sub>H<sub>10</sub>N | 81               |
| 5     | 1340             | Oxalic acid, allyl pentyl ester | C<sub>10</sub>H<sub>14</sub>O<sub>2</sub> | 200              |
| 6     | 715              | 2-methoxy-1,3-dioxolane | C<sub>4</sub>H<sub>10</sub>O<sub>2</sub> | 104              |
| 7     | 578              | 1,3-dioxolane | C<sub>4</sub>H<sub>10</sub>O<sub>2</sub> | 74               |
| 8     | 1312             | 2-benzyl-1,3-dioxolane | C<sub>10</sub>H<sub>14</sub>O<sub>2</sub> | 164              |
| 9     | 1716             | Cyclopentasiloxane, [(2,4,4,6,6,8-heptamethylcyclotetrasiloxan-2-yl) oxy] nonamethyldimethylsiloxane | C<sub>36</sub>H<sub>46</sub>O<sub>10</sub>Si<sub>6</sub> | 652              |
| 10    | 1235             | 2-heptyl-1,3-dioxolane | C<sub>7</sub>H<sub>14</sub>O<sub>2</sub> | 172              |
| 11    | 1612             | 3-ethoxy-1,1,7,7,7-hexamethyl-3,5,5-trisiloxane | C<sub>24</sub>H<sub>34</sub>O<sub>2</sub>S<sub>3</sub> | 562              |
| 12    | 1468             | Malonic acid, bis (2-trimethylsilyl) ester | C<sub>10</sub>H<sub>14</sub>O<sub>2</sub>Si<sub>2</sub> | 304              |
| 13    | 1566             | Acetic acid-[o-(trimethylsilyloxy) pentyl], trimethylsilyl ester | C<sub>12</sub>H<sub>22</sub>O<sub>2</sub>Si<sub>3</sub> | 296              |
| 14    | 1406             | Methyl-alpha.-d-ribofuranoside | C<sub>12</sub>H<sub>22</sub>O<sub>4</sub>S | 164              |
| 15    | 1714             | 3-methylmannoside | C<sub>12</sub>H<sub>22</sub>O<sub>4</sub>S | 194              |
| 16    | 1436             | 2,3,4,5-tetrahydroxypentanal | C<sub>10</sub>H<sub>16</sub>O<sub>4</sub> | 150              |
| 17    | 1359             | Methyl 4-o-methyl-d-arabinopyranoside | C<sub>14</sub>H<sub>22</sub>O<sub>4</sub> | 178              |

**REFERENCES**

1. Okigbo RN, Anuagasi CL, Amadi JE. Advances in selected medicinal and aromatic plants indigenous to Africa. J Med Plants Res 2009;3(2):86-95.
2. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ 2004;6:30-4.
3. Moreira P, Smith MA, Zhu X, Bonda K, Lee HG, Alix G, et al. Since oxidative damage is a key phenomenon in Alzheimer's disease, treatment with antioxidants seems to be a promising approach for slowing disease progression. Oxidative damage and Alzheimer's disease: Are antioxidant therapies useful? Drug News Perspect 2005;18:13-9.
4. Paa-Elizur T, Seviya Z, Leitner-Dagan Y, Elinger D, Roisman LC, Livneh Z. DNA repair of oxidative DNA damage in human cancerogenesis: Potential application for cancer risk assessment and prevention. Cancer Lett 2008;266(1):60-72.
5. Naito Y, Uchiyama K, Yoshikawa T. Oxidative stress involvement in diabetic nephropathy and its prevention by astaxanthin. Oxid Stress Dis 2006;21:235-42.
6. Jain SK. Superoxide dismutase overexpression and cellular oxidative damage in diabetes. A commentary on "Overexpression of mitochondrial superoxide dismutase in mice protects the retina from diabetes-induced oxidative stress". Free Radic Biol Med 2006;41(8):1187-90.
7. Heinecke JW. Mechanisms of oxidative damage of low density lipoprotein in human atherosclerosis. Curr Opin Lipidol 1997;8(5):268-74.
8. Halliwell B. How to characterize a biological antioxidant. Free Radic Res Commun 1999;31(1):1-32.
9. Halliwell B. Antioxidants: The basics - What they are and how to evaluate them. Adv Pharmacol 1997;38:3-20.
10. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160(1):1-40.
11. Wattenberg LW. Chemoprevention of carcinogenesis by minor dietary constituents: Symposium introduction. Pharm Biol 1998;36:6-7.
12. Yang CS, Chung JY, Yang G, Chhabra SK, Lee MJ. Tea and tea polyphenols in cancer prevention. J Nutr 2000;130(2S Suppl):472S-8.
13. Greenberg ER, Baron JA, Tosteson TD, Freeman DH Jr, Beck GI, Bond JH, et al. A clinical trial of antioxidant vitamins to prevent...
colorectal adenoma. Polyp Prevention Study Group. N Engl J Med 1994;331(3):141-7.
14. Wong CJ, Yen GC. Chemopreventive effects of dietary phytochemicals against cancer: mechanisms and metabolites. Phagic acid, monophenol, and their derivatives. Cancer Treat Rev 2012;38(1):76-87.
15. Yang CS, Li G, Yang Z, Guan F, Chen A, Ju J. Cancer prevention by tocopherols and tea polyphenols. Cancer Lett 2013;334(1):79-85.
16. Audry B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dujas F, et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J Ethnopharmacol 2003;84(2-3):131-8.
17. Gaitry CP, Bindia DS, Farhim I, Suba SD. Antimicrobial and antioxidant activities of methanol extract roots of Glyceria glabra and HPLC analysis. Int J Pharm Pharm Sci 2013;5:975-149.
18. Zahir M, Aqil F, Ahmad I. The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. Int J Pharm Pharm Sci 2009;1 Suppl 1:88-95.
19. Niki E. Assessment of antioxidant capacity in vitro and in vivo. Free Radic Biol Med 2010;48(4):503-15.
20. Johnson IT. Phytochemicals and cancer. Proc Nutr Soc 2007;66(2):207-15.
21. Langseth L. Oxidants, Antioxidants, and Disease Prevention. Washington, DC, USA: International Life Sciences Institute Press; 1995.
22. Johnson IT. New approaches to the role of diet in the prevention of cancers of the alimentary tract. Mutat Res 2004;551(1-2):9-28.
23. Silva IK, Soysa P. Evaluation of phytochemical composition and antioxidant capacity of a decoction containing Adenanthera pavonina L. and Thespesia populnea L. Pharmacog Mag 2011;7(27):193-9.
24. Ramli S, Buarathsep S, Tansaringkarn T, Ruangrungsi N. Screening for free radical scavenging activity from ethanolic extract of mimosaceous plants endemic to Thailand. J Health Res 2008;22:55-9.
25. Rahman M, Habib R, Hasan SM, Sayeed MA, Rana S. Cancer prevention by polyphenol, and their derivatives. Cancer Treat Rev 2012;38(1):76-87.
26. Rahman M, Habib R, Hasan SM, Sayeed MA, Rana S. Cancer prevention by polyphenol, and their derivatives. Cancer Treat Rev 2012;38(1):76-87.
27. Heim SC, Guarnier FA, Ferreira DT, Braz-Filho R, Cecchini R, Cecchini AL. Antioxidant activity of Spadheoa campanulata (Bignoniaceae) extracts. Rev Bras Plantas Med Botucatu 2012;14(2):287-92.
28. Aladasanmi AJ, Jwalewa EO, Adehajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, et al. Antimicrobial and antioxidant activities of some Nigerian medicinal plants. Afr J Tradit Complement Altern Med 2006;4(2):173-84.
29. Kumar A, Garg VK, Kumar R, Singh L, Chauhan S. Pharmacognostic study and establishment of quality parameters of leaves of Adhatoda vasica. Linn. J Med Plants Stud 2013;1(3):35-40.
30. Kumar KA, Gousia SK, Anupama M, Latha JN. A review on phytochemical constituents and biological assays of Averrhoa bilimbi. Int J Pharm Pharm Sci Res 2013;3(4):136-9.
31. Gopinath G, Sujesh M, Babu TD. Evaluation of cytotoxic and anti-tumor activity of Phylanthus acidus (L.) Skeels leaf extracts. Int J Nov Res Life Sci 2015;2(2):19-26.
32. Kulkarni AA. Ray of hope for cancer patients. In: Proceedings of the International Seminar on Holistic Management of Cancer, Ayurvedic Education: Series No. 67, 1998. p. 5-11.
33. Hassan R, El-Kadi S, Mostafa S. Effect of some organic acids on some fungal growth and their toxins production. Int J Adv Biol 2015;2:1-11.
34. Küçük HB, Yusufoglu A, Mataraci E, Dösler S. Synthesis and biological activity of new 1,3-dioxolanes as potential antibacterial and antifungal compounds. Molecules 2011;16(8):6806-15.
35. Delcourt A, Mathieu G, Baji H, Kimny T, Flammang M, Compagnon PL. New polyazole derivatives from 2-(2,4-dichlorophenyl)-1,3-dioxolane. Antifungal activity. Structure-activity relationships. Mycopathologia 1997;137(1):27-32.
36. Coleman MD, Zilz TR, Griffiths HR, Woerling EK. A comparison of the apoptotic and cytotoxic effects of hexanedione derivatives on human non-neuronal lines and the neuroblastoma line SH-SY5Y. Basic Clin Pharm Toxicol 2007;102:25-9.
37. Zilz TR, Griffiths HR, Coleman MD. Apoptotic and necrotic effects of hexanedione derivatives on the human neuroblastoma line SK-N-SH. Toxicology 2007;231(2-3):210-4.
38. Baji H, Kimny T, Gasquez F, Flammang M, Compagnon PL, Delcourt A, et al. Synthesis, antifungal activity and structure-activity relationships of 2-(alkyl or aryl)-2-(alkyl or polyazol-1-ylmethyl)-1,3-dioxolane. Eur J Med Pharmacol 2011;6806-15.
39. Aladasanmi AJ, Jwalewa EO, Adehajo AC, Akinkunmi EO, Taiwo BJ, Olorunmola FO, et al. Antimicrobial and antioxidant activities of some Nigerian medicinal plants. Afr J Tradit Complement Altern Med 2006;4(2):173-84.