

# Antioxidant and DNA Damage Protective Effects of *Asparagus racemosus* in Human Colon and Mice Muscle Cells

Girindrababu Venkattappa Jayashree, Puttasiddiah Rachitha, Krishnaswamy Krupashree, Kandikattu Hemanth Kumar, Farhath Khanum\*

Department of Biochemistry and Nanosciences discipline, Defence food research laboratory, Siddartha Nagar, Mysore, Karnataka-570 011, India.

## ABSTRACT

**Introduction:** The present study was designed to investigate the *in vitro* antioxidant and macromolecule damage protective effects of *Asparagus racemosus* water (AWE) and methanolic (AME) fractions of roots. **Methods:** The *in vitro* antioxidant activity of AWE/AME was estimated by free radical scavenging assays. The DNA damage of HT29 and C2C12 cells was analyzed by comet assay. The plasmid DNA damage and protein oxidation were carried out by agarose gel electrophoresis and SDS-PAGE analysis respectively, where as lipid peroxidation was performed by TBARS assay. **Results:** Both the extracts showed scavenging activity with IC<sub>50</sub> values of 417.4 ± 19.5 / 298 ± 13.5, 381 ± 18.2 / 235 ± 11.9, 54.8 ± 2.95 / 31.6 ± 1.52, 28.9 ± 1.73 / 19.7 ± 1.55 µg/mL for DPPH, metal chelating, ABTS and Nitric oxide scavenging activities respectively. Similarly the methanolic extract showed more potent reducing power and total antioxidant activities over water fraction. The AME showed 56.8% and 41.2% protection against H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide) induced DNA damage of HT29 human colon cells and C2C12 murine myoblasts. The extract also showed protection against H<sub>2</sub>O<sub>2</sub> induced plasmid DNA damage, AAPH induced protein oxidation of bovine serum albumin (BSA) and lipid peroxidation of rat hepatic tissue. **Conclusion:** Over all this study showed remarkable antioxidant and macromolecule damage protective effects of *A. racemosus*. The observed biological properties may be attributed to the high content phenols and flavonoids in the methanolic extract *A. racemosus* over water extract.

**Key words:** AAPH, *Asparagus racemosus*, C2C12, HT29, Protein oxidation, Single cell gel electrophoresis.

## INTRODUCTION

The imbalance between oxidants and antioxidants leading to damage of the tissues is known as oxidative stress. The reactive species such as superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH), nitrogen oxide (NO), peroxyxynitrite (ONOO<sup>-</sup>) and hypochlorous acid (HClO) are all the common products of regular metabolism. However under stress conditions the excessive production of these reactive species exerts harmful effects. On the other hand the antioxidants are known to terminate chain reactions initiated by free radicals.<sup>1</sup>

*Asparagus racemosus* known as shatavari belongs to family Liliaceae and is a climbing plant which grows across India. The metabolites of the herb are racemosol, isoflavones, sarsasapogenin glycoside, steroidal sapogenins, ecdysteroids, asparinins, asparosides, curillins, curillosides, shavatarins, fructo-oligosaccharides and fatty acids.<sup>2-5</sup> In the traditional system of medicine the herb is used as demulscent, diuretic, aphrodisiac and galactogogue.<sup>6</sup> *A. racemosus* is also used to prevent ageing, to increase longevity, to improve mental function and to boost immunity.<sup>7,8</sup> Further reports indicate the pharmacological activities of *A. racemosus* root that include antiulcer and antidiabetic activities.<sup>9,10</sup>

In the present investigation we aimed to study the free radical induced *in vitro* antioxidant scavenging activity and macromolecule damage protective effects of *A. racemosus* aqueous and methanolic extract against H<sub>2</sub>O<sub>2</sub> induced

### \*Corresponding author:

Dr. Farhath Khanum

Biochemistry and Nanosciences discipline,  
Defence food research laboratory,  
Siddartha Nagar, Mysore,  
Karnataka-570 011, India.  
E-mail: farhathkhanum@gmail.com

DOI: 10.5530/pj.2015.3.7

DNA damage of human colon and mice muscle cells, AAPH induced protein and hepatic tissue damage.

## MATERIALS AND METHODS

### Chemicals and reagents

Hydrogenperoxide was procured from Merck (Bangalore, India), gallic acid, catechin, AAPH, BSA, DMEM, fetal bovine serum, penicillin, streptomycin solution from Sigma (Bangalore, India), horse serum from Hyclone (Bangalore, India), whereas other chemicals used were of analytical grade and procured from Hi-Media (Bangalore, India).

### Preparation of methanolic extract

The methanolic extract of *Asparagus racemosus* (AME) was prepared as described earlier.<sup>11</sup> Briefly the roots were washed, cut into small pieces, shade dried and powdered. The powdered material was extracted using methanol and concentrated under reduced pressure using rotary flash evaporator (Rotovac, Schwabach, Germany). Whereas the aqueous extract (AWE) was prepared by maceration of *A. racemosus* root powder with water, followed by filtration and lyophilization (Lyolab, Hyderabad, India).

### Estimation of total polyphenol content

Total polyphenol content was determined using Folin–Ciocalteu reagents where as gallic acid was used as a standard. Gallic acid 20–100 µg was added to the test tubes and the volume was made up to 3 mL using distilled water. *A. racemosus* extracts (1 mg/mL) were taken in the range of 100–1000 µg, and the volume was made up to 3 mL using distilled water and 0.5 mL of Folin–Ciocalteu reagent was added to all the tubes followed by incubation for 6 mins. To this mixture 2 mL of 7% sodium carbonate was added and the absorbance was measured at 650 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed in terms of µg (gallic acid equivalents) GAE/mg of extract.

### Determination of flavonoid content

The plant extracts/catechin as standard (1mg/mL) were added at different concentrations and made to one mL with distilled water. To this 75 µL of 5% NaNO<sub>2</sub> and 150 µL of 10% AlCl<sub>3</sub> were added and incubated for a period of 10 mins. To this mixture 500 µL of 1M NaOH and 775 µL of distilled water was added and the reaction was measured at 510 nm against reagent blank. The results were expressed in terms of µg (catechin equivalents) CE/mg of extract.

## *In vitro* antioxidant scavenging activities

### Total antioxidant activity

The total antioxidant activity of AWE and AME were evaluated spectrophotometrically. The plant extracts 1 mg/mL or gallic acid as standard (1 mg/mL) were added to the tubes and finally the volume was made to 0.3–mL using distilled water followed by 3 mL of reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 mins. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank and the results were expressed in terms of µg GAE/mg of extract.

### Reducing power

The total reducing power was evaluated by using ascorbic acid as standard (1mg/mL) and plant extracts (1 mg/mL) added in different dilutions to the test tubes and the volume was made up to 1 mL using distilled water. To this 2.5 mL of PBS and 1% potassium ferric cyanide were added and the reaction mixtures were incubate at 50°C for 20 mins. Finally to the tubes 2.5 mL of 10% TCA and 0.1% of 0.5 mL ferric chloride and 2.5 mL of distilled water were added. The absorbance of each sample was measured at 700 nm using a spectrophotometer and the results were expressed in terms of µg (ascorbic acid equivalents) AAE/mg of extract.

### DPPH radical Scavenging assay

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radical with violet color and the scavenging of DPPH radicals generate yellow color. Based on this principle herbal extracts/bioactive compound have been checked for their free radical scavenging activity. The water/methanolic extracts and BHA (1 mg/mL) as standard were added and the volume is made up to 3 mL using methanol. To this freshly prepared DPPH solution was added and the tubes were incubated at room temperature for 45 mins. The absorbance was read at 515 nm and the results were expressed in terms of IC<sub>50</sub> values.

### Metal chelating activity

The metal chelating activity was determined using ferrozine reagent with EDTA as standard. The extracts (1 mg/mL) and EDTA (0.1 mg/mL) were taken in different concentrations in the test tubes and the volume was made up to 3 mL using distilled water. To this 50 µL of ferric chloride and 2 mL of ferrozine were added and incubated for 10 mins. at room

temperature. The absorbance was measured at 562 nm and the results were expressed in terms of IC<sub>50</sub> values.

#### *ABTS radical scavenging activity*

The ABTS radical scavenging activity was evaluated using ascorbic acid as standard. Prior to the assay the ABTS reagent was prepared by mixing 7 mM ABTS and 2.45 mM potassium per sulphate and the solution was diluted with ethanol to get a blue-green chromophore with an absorbance of 0.700 ± 0.025 at 734 nm. The extracts (1 mg/mL) were added in different concentrations and the volume was made up to 1 mL using distilled water. To this 1 mL of ABTS reagent was added and the samples were incubated at room temperature for 6 mins. The absorbance of each sample was measured at 734 nm and the results were expressed in terms of IC<sub>50</sub> values.

#### *Nitric oxide scavenging activity*

This assay was performed as described by Sreejayan and Rao (1997).<sup>12</sup> Briefly, sodium nitroprusside (10 mM) in PBS was mixed with different concentrations of extracts/standard and incubated at room temperature for 150 mins. The reaction mixture without the sample/standard served as the control. After incubation, 0.5 mL of Griess reagent [1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-naphthyl) ethylenediamine HCl] was added and the absorbance was recorded at 546 nm. L-ascorbic acid was used as a reference standard and the results were expressed in terms of IC<sub>50</sub> values.

#### *Hydroxyl radical scavenging activity*

This assay was performed according to the method of Kunchandy and Rao (1990).<sup>13</sup> The Fenton reaction was initiated using Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> to generate hydroxyl radicals which degrades DNA deoxyribose. The reaction mixture composed of 2-deoxy-2-ribose (2.8 mM); KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (20 mM, pH 7.4); FeCl<sub>3</sub> (100 μM); EDTA (100 μM); H<sub>2</sub>O<sub>2</sub> (1.0 mM); ascorbic acid (100 μM) and different concentrations of (0-200 μg/mL) of extracts/standard (ascorbic acid). After incubation for 1 h at 37°C, 0.5 mL of the reaction mixture was added to 1 mL of 2.8% trichloroacetic acid. Finally 1 mL of 1% TBA was added and the mixture was incubated at 90°C for 15 mins. The developed color product was measured at 532 nm and the results were expressed in terms of IC<sub>50</sub> values.

### **Macromolecule damage protective activity**

#### *Plasmid DNA damage nick assay*

The protective effect of *A. racemosus* methanolic extract

against H<sub>2</sub>O<sub>2</sub> induced DNA damage was studied using pRSET-A plasmid DNA as described by Russo et al. (2000).<sup>14</sup> Plasmid DNA (350 ng) in 10 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), H<sub>2</sub>O<sub>2</sub> was added at a final concentration of 10 mM with or without ARE (2.5, 5 and 10 μg/mL)/gallic acid (5 μg/mL) and incubated for a period of 5 mins at room temperature. Plasmid DNA without any treatment was used as control. After due incubations, the plasmid DNA was electrophoresed on 1% agarose gel. Finally the gel was stained with ethidium bromide and photographed using gel documentation system (Syngene, Cambridge, UK).

#### *Cell culture*

HT29 Human colon carcinoma cells and C2C12 murine myoblasts were procured from National Centre for Cell Sciences, Pune, India.

#### *HT29 Human colon carcinoma cells*

HT29 cells (4 X 10<sup>5</sup> cells/mL) were grown in 75 cm<sup>2</sup> flasks supplemented with Dulbecco's modified eagle's medium (DMEM) and 10% fetal bovine serum, containing, penicillin and streptomycin solution in humidified atmosphere at 37 °C with 5% CO<sub>2</sub>.

#### *C2C12 murine muscle cells*

C2C12 murine muscle cells (4 X 10<sup>5</sup> cells/mL) were grown in 75 cm<sup>2</sup> flasks supplemented with 1:1 mixture of DMEM and 10% fetal bovine serum, containing, penicillin and streptomycin solution in humidified atmosphere at 37 °C with 5% CO<sub>2</sub> for 5 days. The differentiation of muscle cells to myoblasts was carried out by changing the cells to DMEM with 2 % horse serum.

#### *Single cell gel electrophoresis*

Once the confluency reached HT29 and C2C12 cells were collected by trypsinization and washed twice with PBS to remove serum and media. The cells were treated with 200 μM H<sub>2</sub>O<sub>2</sub> with or without pretreatment of *A. racemosus* (100 μg/assay). The DNA damage and the protective effect of AME were measured by alkaline comet assay as described.<sup>15</sup> The cells were mixed with 100 μL of 0.7% (w/v) low melting agarose (LMA) and pipetted on to the frosted slides pre-coated with 1.0% (w/v) normal melting agarose. After solidification, the slides were covered with another 100 μL of 0.7% (w/v) LMA and immersed in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl buffer, 0.1% SDS and 1% Triton X-100 and 10% DMSO;

pH 10.0) for 90 mins. After lysis the slides were transferred to electrophoresis tank with unwinding buffer (3 M NaOH, 10 mM EDTA; pH 13.0) for DNA unwinding. Later the slides were run with an electric current of 25 V/300 mA for 20 mins. Followed by unwinding the slides were washed twice with neutralizing buffer (0.4 M Tris– HCl; pH 7.5). Finally the DNA in the agarose gels was stained with ethidium bromide (20 µg/mL) and the DNA damage was observed and photographed using fluorescence microscope (Olympus, Japan) equipped with Cool SNAP® Pro color digital camera. The tail length and per cent inhibition of DNA damage with AME treatment was determined using Image Pro® plus software.

### Protein oxidation

The oxidation of bovine serum albumin (BSA) was carried using AAPH, which is an initiator of peroxy radicals upon decomposition with water.<sup>16</sup> BSA (5 µg) was dissolved in phosphate buffer (pH 7.3) and incubated for 2 h with or without 40 mM AAPH and in the presence or absence of AME. Finally the protein samples were subjected to SDS-PAGE electrophoresis. The gels were stained with 0.15% coomassie brilliant blue R-250 and the amount of protein damage was quantified by measuring the density of each band using NIH Image J software.

### Lipid peroxidation

The lipid peroxidation was spectrophotometrically estimated in terms of thiobarbituric acid reactive substances (TBARS) formed in the liver homogenate treated with AAPH radicals as described by Wright et al. (1981).<sup>17</sup> Liver tissues were collected from 3-4 months old male Wistar albino rats weighing 120 ± 12.5 g and washed with 0.95% NaCl solution. Liver homogenates were prepared in ice-cold 3 mM tris buffer containing 250 mM sucrose and 0.1

mM EDTA (pH 7.4). To 0.5 mL of liver homogenates 0-100 µL of AME was added and made up to 1 mL with phosphate buffer (0.1 M, pH 7.4). Lipid peroxidation was initiated by treatment with 1 mL of 200 µM AAPH followed by incubation at 37°C for 2 h and the reaction was terminated by addition of 1.0 mL of TCA (10%, w/v). Finally 1.0 mL of TBA (0.67%, w/v) was added and the tubes were placed in a boiling water bath for 20 mins. Samples were centrifuged at 2,500 g for 10 mins. and the malondialdehyde formed in each sample was assessed by measuring the absorbance at 535 nm against a reagent blank. BHA was used as a standard antioxidant.

## RESULTS

### Total polyphenolic and flavonoid content

The water and methanolic fractions of *A. racemosus* showed 146 ± 8.2 and 246 ± 11.8 µg GAE/mg total polyphenols wher as the flavonoid content was found to be 113 ± 6.8 and 154 ± 7.4 µg CE/mg respectively. These results shows that methanolic fraction possess high content of polyphenols and flavonoids over water fraction of *A. racemosus* (Table 1).

### In vitro antioxidant and free radical scavenging activity

The water and methanolic fractions of *A. racemosus* were further analysed for their antioxidant activities by an array of *in vitro* antioxidant assays. Our results demonstrates that AWE/AME exhibits total antioxidant and reducing power activities of 229 ± 12.3/349 ± 17.7 µg GAE/mg and 162 ± 9.3/230 ± 10.3 µg AAE/mg respectively. Whereas the DPPH, metal chelating, ABTS, NO and hydroxyl radical scavenging activities of AWE/AME falls in the range of

**Table 1: Demonstrate that methanolic extract of *A. racemosus* inhibits the free radicals more effectively compared with the water fraction.**

Assay	Water extract (AWE)	Methanolic extract (AME)
Total polyphenolic content	146 ± 8.2 µg GAE/mg	246 ± 11.8 µg GAE/mg
Total flavonoids	113 ± 6.8 µg CE/mg	154 ± 7.4 µg CE/mg
Total antioxidant activity	229 ± 12.3 µg GAE/mg	349 ± 17.7 µg GAE/mg
Reducing power	162 ± 9.3 µg AAE/mg	230 ± 10.3 µg AAE/mg
DPPH	417.4 ± 19.5 IC50 (µg/ml)	298 ± 13.5 IC50 (µg/ml)
Metal chelating	381 ± 18.2 IC50 (µg/ml)	235 ± 11.9 IC50 (µg/ml)
ABTS	54.8±2.95 IC50 (µg/ml)	31.6±1.52 IC50 (µg/ml)
NO scavenging	28.9±1.73 IC50 (µg/ml)	19.7±1.55 IC50 (µg/ml)
Hydroxyl radical scavenging activity	312±16.55 IC50 (µg/ml)	251±12.35 IC50 (µg/ml)
Anti-Lipid peroxidation	514±23.75 IC50 (µg/ml)	437 ± 22.7 IC50 (µg/ml)

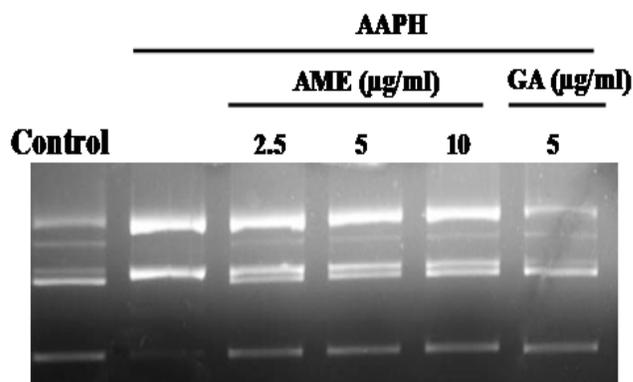
IC<sub>50</sub> values of 417.4 ± 19.5/298 ± 13.5, 381 ± 18.2/235 ± 11.9, 54.8 ± 2.95/31.6 ± 1.52, 28.9 ± 1.73/19.7 ± 1.55, 312 ± 16.55/251 ± 12.35 µg/ml respectively. These results demonstrate that methanolic extract of *A. racemosus* inhibits the free radicals more effectively compared with the water fraction (Table 1).

**Macromolecule damage protective effects of *A. racemosus***

Based on the above assays it is confirmed that methanolic fraction of *A. racemosus* is more potent and the same was used to analyze chemicals (H<sub>2</sub>O<sub>2</sub>/AAPH) induced macromolecules (DNA, protein and lipid) damage protective effects.

**Protective effects of AME against H<sub>2</sub>O<sub>2</sub> induced Plasmid DNA damage**

The methanolic extract was evaluated for its protective effect on H<sub>2</sub>O<sub>2</sub> induced damage of pRSET-A plasmid DNA and analyzed by agarose gel electrophoresis (Figure 1). Negative control (lane-1) showed two bands on agarose gel electrophoresis wherein the faster moving band corresponded to the native supercoiled circular DNA (ScDNA) and the slow moving faint band corresponded to the open circular form (OcDNA). Whereas DNA challenged with H<sub>2</sub>O<sub>2</sub> (lane 2) resulted in conversion of supercoiled DNA to linear form indicating the hydroxyl

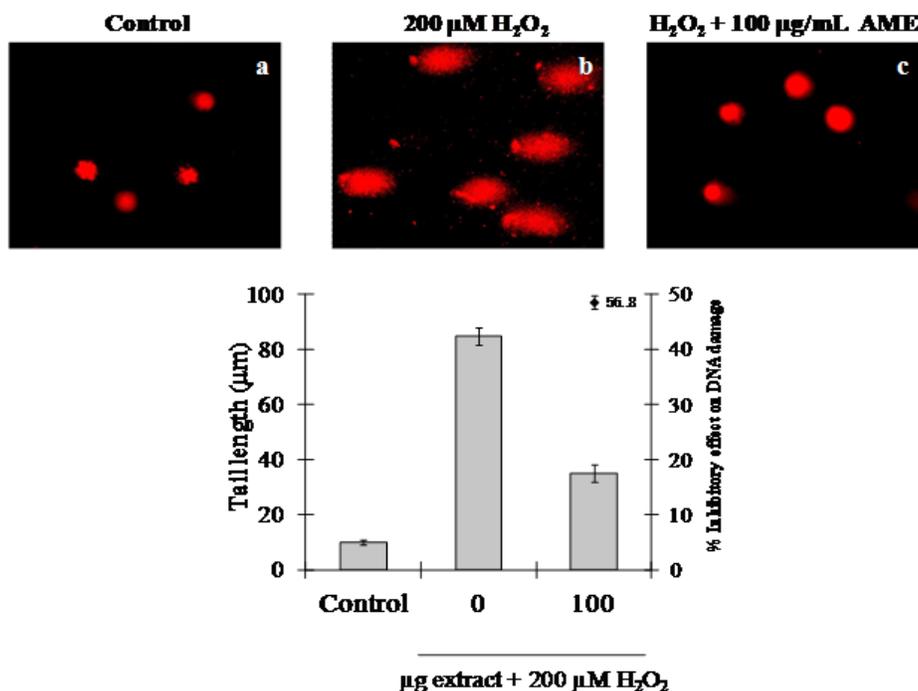


**Figure 1: The protective effect of *A. racemosus* on H<sub>2</sub>O<sub>2</sub> induced Plasmid DNA damage analysis by agarose gel electrophoresis**

radical mediated DNA damage. In the presence of 2.5, 5, 10 µg/mL of *A. racemosus* and 5 µg/mL of gallic acid treatment significant protection to the damage of ScDNA was observed in a dose dependent manner (lanes 3-6).

**AME protects H<sub>2</sub>O<sub>2</sub> induced colon and muscle cell damage**

Further the DNA damage protective effects of *A. racemosus* was verified by single cell gel electrophoresis assay against H<sub>2</sub>O<sub>2</sub> induced DNA damage of HT29 human colon and C2C12 murine muscle cells. The DNA damage pattern of HT 29 and C2C12 cells was observed in the form of comets



**Figure 2 A: The protective effect of *A. racemosus* on H<sub>2</sub>O<sub>2</sub> induced DNA damage of HT29 human colon cells by single cell gel electrophoresis assay. A. control cells B. 200 µM H<sub>2</sub>O<sub>2</sub> treated cells C. 200 µM H<sub>2</sub>O<sub>2</sub> + 100 µg/reaction AME treated cells**

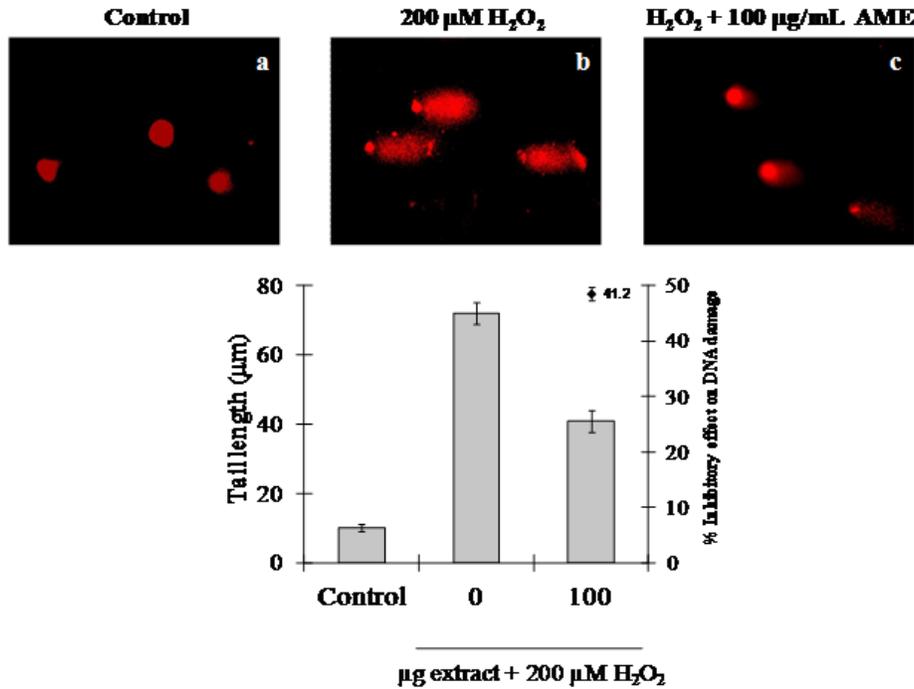


Figure 2B: The protective effect of *A. racemosus* on H<sub>2</sub>O<sub>2</sub> induced DNA damage of C2C12 murine muscle cells by single cell gel electrophoresis assay. A. control cells B. 200 μM H<sub>2</sub>O<sub>2</sub> treated cells C. 200 μM H<sub>2</sub>O<sub>2</sub> + 100 μg/reaction AME treated cells.

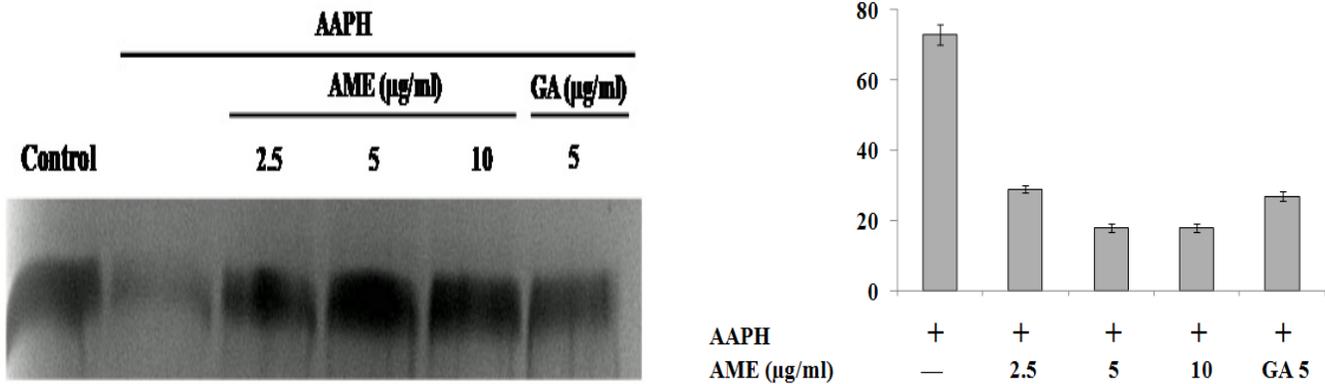


Figure 3: The protective effect of *A. racemosus* on AAPH induced protein oxidation of BSA analysed by polyacrylamide gel electrophoresis. The densitometric analysis of protein oxidation by NIH Image J software.

as shown in (Figure 2a and 2b), which was found to be 85 μm and 72 μm respectively with H<sub>2</sub>O<sub>2</sub> challenge. Whereas pretreatment of HT29 and C2C12 cells showed 56.8% and 41.2% DNA damage inhibitory activity respectively. These observations demonstrates the protective effect of *A. racemosus* against colon and muscle cell damage.

### AME protects AAPH induced protein oxidation

The protective effect of methanolic fraction of *A. racemosus* was analyzed by BSA induced protein oxidation assay against an azo compound AAPH which generates peroxy radicals. After the reaction the densitometric pattern

analysis of individual protein samples ran by polyacrylamide gel electrophoresis showed that AAPH induces 75% protein oxidation. Whereas the AAPH induced protein oxidation was dose dependently inhibited by pretreatment of BSA with *A. racemosus* (Figure 3).

### Lipid peroxidation inhibitory activity of AME

The lipid peroxidation activity of AWE/AME was found to be IC<sub>50</sub> of 514 ± 23.75/437 ± 22.7 μg/mL respectively, which further confirms that methanolic fraction is a potent lipid peroxidation scavenger compared to that of water fraction of *A. racemosus* (Table 1).

## DISCUSSION

Herbs have been used in traditional food system due their nutritional and health benefits. *Asparagus racemosus* is a well known food plant consumed in most parts of the world. *A. racemosus* roots, the edible part of the plant are used in salads, vegetable dishes and soups. It has gained a lot of significance in Indian traditional system of medicine. *A. racemosus* possess an array of compounds that include polyphenols, flavonoids, oligosaccharides, aminoacids, sulphur containing acids and steroidal saponins.<sup>18</sup> these compounds have been reported as radical scavengers due to their antioxidant activity.<sup>19</sup>

Phenolic and flavonoid compounds are reported as antioxidant principles which can alleviate various stress induced disorders such as diabetes, cardiovascular diseases and cancer.<sup>20</sup> It possesses polyphenols such as quercetin-3-glucuronoids, racemosol,<sup>21,22</sup> and flavonoids such as kaempferol, rutin, hyperoside and isoflavone; 8-methoxy-5, 6, 4-trihydroxy isoflavone-7-0-beta-D-glucopyranoside.<sup>2,23,24</sup> Earlier reports demonstrated that these compounds possess anti-inflammatory, antiosteoclastogenic, cytotoxic, immunomodulatory, anti-cancer and antibacterial activities.<sup>25-27</sup>

Herbal extracts have been used as food additives to add health benefits along with nutritional supplementation, these are called functional foods. Hence there is a growing interest across the globe in the commercialization of health foods. The health benefits of food additives and their phytochemicals has to be investigated to give a clear idea about the functional properties of foods.<sup>28</sup> described the add-on benefits of functional foods against wide range of radical mediated cell damage. The radical scavenging ability of *A. racemosus* was evaluated by an array of free radical assays such as DPPH, metal chelating, ABTS, NO, hydroxyl radical scavenging activities. These assays are based on the principle of free radical generation.<sup>1,29</sup> Here we observed that the methanolic fraction of *A. racemosus* exerts potent radical scavenging over water fraction which is due to its hydrogen ion donation, electron transfer, chain termination and nitrite ions reduction activities. This confer that the recovery and composition of phytochemicals varies based on the polarity of the solvents used for the extraction process. The functional properties of extracted solvents also differ based on the composition of phytochemicals recovered.

The macromolecules such as DNA, proteins and lipids damage have been observed in diabetics, cancer and in ageing.<sup>30-32</sup> Several chemicals/radicals were used to evaluate

their toxicity at cellular level and the protective activity of functional foods against the toxicity was evaluated routinely by comet assay.<sup>33,34</sup> In our present study the protective effect of *A. racemosus* against H<sub>2</sub>O<sub>2</sub> challenged DNA in HT29 human colon, C2C12 mice muscle cells and pRSET A plasmid was analyzed by single cell gel electrophoresis assay and agarose gel electrophoresis. The results demonstrate that AME exerts DNA damage protective effects. Similarly *A. racemosus* also showed protective effects against AAPH induced oxidation of bovine serum albumin protein. The observed results are in accordance with our recent study which shows that *Cyperus rotundus* exhibits DNA damage and protein oxidation inhibitory activity against H<sub>2</sub>O<sub>2</sub>/AAPH induced oxidation of macromolecules.<sup>35</sup>

Malonaldehyde (MDA) is a degradation product that is generated by oxidative degradation of polyunsaturated fatty acids in the cell membranes which is an important cause for cellular damage and cell membrane destruction.<sup>36</sup> Estimation of lipid peroxidation has been used as a marker for the oxidative stress. The reaction of MDA with TBA has been widely adopted as a sensitive assay for the lipid peroxidation.<sup>37</sup> In our present study we found that AME significantly inhibits the peroxidation of lipids. Our observed antilipidperoxidation activity is in line with earlier report which also evaluated the lipid peroxidation inhibitory activity of *Withania somnifera* root extracts.<sup>38</sup>

## CONCLUSION

The present study demonstrates that methanolic extract is a potent scavenger of free radicals over water extract of *A. racemosus* as analyzed by an array of *in vitro* antioxidant assays. The methanolic fraction showed inhibitory activity against H<sub>2</sub>O<sub>2</sub> induced plasmid DNA damage and AAPH induced protein and lipid peroxidation. Further *A. racemosus* methanolic extract also inhibited H<sub>2</sub>O<sub>2</sub> induced human colon and murine muscle cell DNA damage as verified by single cell gel electrophoresis assay. Overall our results demonstrate the antioxidant and macromolecule damage protective effects of *A. racemosus* methanolic extract. However, further phytochemical analysis and *in vivo* studies are required to identify the specific compounds and to better clarify the biological activity of *Asparagus racemosus*.

## CONFLICT OF INTEREST

We declare that we do not have any conflict of interest.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. HV Batra, Director, Defence Food Research Laboratory, Mysore, for providing all the necessary facilities, constant guidance and encouragement during this investigation.

## REFERENCES

- Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of Cellular and Molecular Medicine* 2010; 14(4): 840-60.
- Saxena VK, Chourasia S. A new isoflavone from the roots of *Asparagus racemosus*. *Fitoterapia*. 2001; 72(3): 307-9.
- Patricia Hayes Y, Aisyah Jahidin H, Reg Lehmann, Kerry Penman, William Kitching James J, De Voss. Asparinins, asparosides, curillins, curillosides and shavatarins: structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of *Asparagus racemosus*. *Tetrahedron Letters*. 2006; 47(49): 8683-7.
- Sharma P, Chauhan PS, Dutt P, Amina M, Suri KA, Gupta BD, et al. A unique immuno-stimulant steroidal saponin acid from the roots of *Asparagus racemosus*. *Steroids*. 2011; 76(4): 358-64.
- Thakur M, Connellan P, Deseo MA, Morris C, Praznik W, Loeppert R, et al. Characterization and *in vitro* immunomodulatory screening of fructo-oligosaccharides of *Asparagus racemosus* Willd. *International Journal of Biological Macromolecules* 2012; 50(3): 77-81.
- Potduang B, Meeploy M, Giwanon R, Benmart Y, Kaewduang M, Supatanakul W. Biological activities of *Asparagus racemosus*. *African Journal of Traditional, Complementary and Alternative Medicines* 2008; 5(3): 230-70.
- Parihar MS, Hemnani T. Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus*. *Journal of Neural Transmission* 2004; 111(1): 1-12.
- Gautam M, Saha S, Bani S, Kaul A, Mishra S, Patil D, et al. Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity for immune adjuvant potential. *Journal of Ethnopharmacology* 2009; 121(2): 241-70.
- Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of *Asparagus racemosus* Willd and *Withania somnifera* Dunal in rats. *Annals of the New York Academy of Sciences* 2005; 1056(1): 261-78.
- Hannan JM, Marenah L, Ali L, Rokeya B, Flatt PR, Abdel-Wahab YH. Insulin secretory actions of extracts of *Asparagus racemosus* root in perfused pancreas, isolated islets and clonal pancreatic beta-cells. *Journal of Endocrinology* 2007; 192(1): 159-68.
- Jayashree GV, Rachitha P, Krupashree K, Hemanth Kumar K, Farhath Khanum. Phytochemical analysis of methanolic extract of roots of *Asparagus racemosus* (Shatavari). *International Journal of Pharma and Bio sciences* 2013; 4(4): 250-4.
- Sreejayan, Rao MNA. Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology* 1997; 49(1): 105-7.
- Kunchandy, Rao Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics* 1990; 58(3): 237-40.
- Russo A, Acquaviva R, Campisi A, Sorrenti V, Di Giacomo C, Virgata G, et al. Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell biology and toxicology* 2000; 16(2): 91-8.
- Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*. 1988; 175(1): 184-91.
- Mayo JC, Tan DX, Sainz RM, Natarajan M, Lopez-Burillo S, et al. Protection against oxidative protein damage induced by metal-catalyzed reaction or alkylperoxyl radicals: comparative effects of melatonin and other antioxidants. *Biochimica et Biophysica Acta* 2003; 1620(1): 139-50.
- Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Archives of Biochemistry and Biophysics* 1981; 206(2): 296-304.
- Negi JS, Singh P, Joshi GP, Rawat MS, Bisht VK. Chemical constituents of asparagus. *Pharmacognosy Reviews* 2010; 4(8): 215-20.
- Rubio L, Motilva MJ, Romero MP. Recent advances in biologically active compounds in herbs and spices: A review of the most effective antioxidant and anti-inflammatory active principles. *Critical Reviews in Food Science and Nutrition* 2013; 53(9): 943-53.
- Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity* 2009; 2(5): 270-80.
- Rastogi RP, Mehrotra BN. New Delhi: Central Drug Research Institute, Lucknow and Publications & Information Directorate. *Compendium of Indian Medicinal Plants* 1969; 49-50.
- Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): A review. *Asian Pacific Journal of Tropical Disease* 2013; 3(3): 242-51.
- Sharma SC. Constituents of the fruits of *Asparagus racemosus* Willd. *Pharmazie* 1981; 36(10): 709.
- Ahmad S, Ahmad S, Jain PC. Chemical examination of Satavari (*Asparagus racemosus*). *Bull Medico Ethnobotanical Research PC*. 1991; 12(3/4): 157-60.
- Pang JL, Ricupero DA, Huang S, Fatma N, Singh DP, Romero JR, et al. Differential activity of kaempferol and quercetin in attenuating tumor necrosis factor receptor family signaling in bone cells. *Biochemical Pharmacology* 2006; 14; 71(6): 818-26.
- Garcia-Mediavilla V, Crespo I, Collado PS, Esteller A, Sanchez-Campos, Tunon MJ, et al. The anti inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappa B pathway in Chang Liver cells. *European Journal of Pharmacology* 2007; 557(2): 221-90.
- Paszkievicz M, Budzynska A, Rozalska B, Sadowska B. The immunomodulatory role of plant polyphenols. *Postepy Hig Med Dosw*. 2012; 66: 637-46.
- Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 2010; 4(8): 118-26.
- Gülçin I. Antioxidant activity of food constituents: an overview. *Archives of toxicology* 2012; 86(3): 345-91.
- Maynard S, Schurman SH, Harboe C, De Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis* 2009; 30(1): 2-10.
- Speakman JR, Selman C. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *Bioessays* 2011; 33(4): 255-9.
- Pazdro R, Burgess JR. Differential effects of  $\alpha$ -tocopherol and N-acetyl-cysteine on advanced glycation end product-induced oxidative damage and neurite degeneration in SH-SY5Y cells. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 2012; 1822(4): 550-6.
- Han KC, Wong WC, Benzie IF. Genoprotective effects of green tea (*Camellia sinensis*) in human subjects: results of a controlled supplementation trial. *British Journal of Nutrition* 2011; 105(02): 171-9.
- Del Bo' C, Riso P, Campolo J, Moller P, Loft S, Klimis-Zacas D, et al. A single portion of blueberry (*Vaccinium corymbosum* L) improves protection against DNA damage but not vascular function

- in healthy male volunteers. *Nutrition Research* 2013; 33(3): 220-7.
35. Hemanth Kumar K, Razack S, Ilaiyaraja N, Khanum F. Phytochemical analysis and biological properties of *Cyperus rotundus* L. *Industrial Crops and Products* 2014; 52: 815-26. 10.1016/j.indcrop.2013.11.040b.
36. Kubo S. Routes of formation of toxic consequences of lipid oxidation products in foods. *Free Radical Biological Medicines* 1992; 12(1): 63–81.
37. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 1978; 95(2): 351–8.
38. Pal A, Naika M, Khanum F, Bawa AS. *In-Vitro* Studies on the Antioxidant Assay Profiling of Root of *Withania somnifera* L. (Ashwagandha) *Dunal* 2012; 77(2): 95-101.