# Antiproliferative Effect of Antioxidative Free and Bound Phenolics from *Andrographis serpyllifolia*

**Smitha Jayaram, and Shylaja M. Dharmesh***

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India

## ABSTRACT

**Background:** *Andrographis serpyllifolia*, used as a medicinal plant in traditional practices in India, where in cancer patients are treated using the leaves of *A. serpyllifolia.* The crude extracts of *A. serpyllifolia* not only contain a wide variety of phenolic compounds but also show an excellent antioxidant activity. Therefore, this plant might be a good candidate for further development for its antioxidant remedies. However, the biological activities of the phenolic extracts of *A. serpyllifolia* on cancer have not been studied to date. **Objective:** To investigate the antioxidant and antiproliferative properties of the phenolics of *A. serpyllifolia.* **Materials and methods:** Free and (ASFP) and bound (ASBP) phenolics of *A. serpyllifolia* were isolated and determined antioxidant and antiproliferative abilities that are required for anticancer properties. **Results:** Individual phenolic constituents present in each of these fractions and their precise contribution to both antioxidant and antiproliferative activities were determined to justify the traditionally observed result of anticancer properties. Treatment of HeLa cells with ASFP and ASBP showed antiproliferative activity with increased malondialdehyde (MDA) as well as decreased levels of reduced glutathione (GSH). The present experimental data suggest that components within the ASFP may have inherent properties that suppress cancer cell proliferation. The phenolic fractions were also screened for their potential antioxidant activities using DPPH, reducing power, DNA protection, inhibition of lipid peroxidation and protein carbonyls model systems. ASFP exhibited highest antioxidant activity in all the model systems employed to study antioxidant activity. The positive correlation between polyphenolic content of *A. serpyllifolia* to its antioxidant activity was seen. **Conclusion:** Potent antiproliferative and DNA protective activity of ASFP and ASBP may contribute significantly against cancer pathogenesis.

**Key words:** Antioxidant; Antiproliferative; Glutathione; Malondialdehyde; Phenolic fractions

***Address for correspondence:** Dr. Shylaja M Dharmesh, Department of Biochemistry and Nutrition,
Central Food Technological Research Institute, Mysore 570020, India.

**Phone:** 91+0821+2514876, Fax: 91+0821+2517233

**E-mail:** biolab4cancer@gmail.com

**DOI:** 10.5530/ax.2011.3.8

## INTRODUCTION

*Andrographis serpyllifolia,* an edible, railing and rooting herb known for its traditional medicinal properties. The herb belongs to the family *Acanthaceae* is widely distributed throughout Deccan and carnatic region of south India [1] and China. Although the related species *A. paniculata* is well characterized, only a very few studies report the presence of serpyllin, apigen, 7,4'- dimethyl ether and tectochrysin compounds[2,3] and, their implications on bioactivity is not clearly understood. Andrographolide (AG) has been reported as one of the potential active component and believed to be responsible for various bioactivities.[4-6] We reported for the first time, the antimetastatic property of pectic polysaccharide[7] and antioxidant activities of crude extracts of *A. serpyllifolia.*[8]

Excessive generation of free radicals is implicated in an ever growing number of disease conditions, including cancer, atherosclerosis, and neurodegenerative diseases.[9,10] Over the past decades, much research was aimed to counterbalance the adverse effects of free radicals by complementing the endogenous antioxidant defense system with dietary antioxidants.[11] In the search of plants as a source of natural antioxidants, some medicinal plants, vegetables, and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few years.[12,13] Attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical and food processing applications.

The roles of herbal extracts in disease prevention and cure have been attributed to antioxidant properties of their constituents - broadly termed phenolic compounds. Based on the survey of traditional practices, where in cancer patients are treated using the leaves of *A. serpyllifolia,* the current study was undertaken to investigate the antioxidant and antiproliferative properties of phenolics of *A. serpyllifolia*.

Current study therefore addresses (1) Isolation of free and bound phenolics, since phenolics have been shown to be associated with various biomolecules such as proteins, carbohydrates etc, in addition to existence in free form and to examine the total phenolic contents; (2) identification and quantification of phenolic acids by high-performance liquid chromatography (HPLC); (3) determination of antioxidant activity using multi-mechanistic assays; (4) determination of antiproliferative activity, and (5) evaluation of the relationship between phenolic contents and antioxidant activities. Such a studies provide impact on the health-promoting activity of herbal extracts.

## MATERIALS AND METHODS

### *Plant material*

*A. serpyllifoila* Wt. Ie found locally in Chamundi hills, near ponds and pools of Mysore, Karnataka, India were collected, identified by comparison with authenticated specimens and deposited at the Herbarium of the Department of Studies in Botany, University of Mysore, Mysore, India. Leaves were used as source for the study. Information about its use as traditional health beneficial source, both as food and medicine was collected from tribals and local Ayurveda doctors in Mysore district, India.

### *Isolation of free and bound phenolic fractions of* A. serpyllifolia

Free phenolics were isolated according to the method of Ayumi et al.[14] Two grams of defatted *A. serpyllifolia* powder was extracted with 70% ethanol (4 × 50 mL, 1 h each), the supernatants were obtained by centrifugation at 600 × g for 10 min at room temperature; concentrated, and the pH was adjusted in the range of 2-3 with 4 M HCl. Phenolic acids were separated by ethyl acetate phase separation (5 × 50 mL), and the pooled fractions were treated with anhydrous di sodium sulfate to remove moisture, filtered (Whatman grade No. 1 filter paper) and evaporated to dryness. Phenolic acids were reconstituted in methanol and designated as *Andrographis serpyllifolia* Free Phenolic Fraction (ASFP).

Bound phenolics were extracted according to the method of Nordkvist et al.[15] Two grams of AS powder was extracted with 4 × 50 mL of 70% ethanol (4 × 50 mL, 1 h each). The dried samples were extracted with 2 × 100 mL of 1 M sodium hydroxide containing 0.5% sodium borohydride under nitrogen atmosphere for 2 h and the clear supernatants were collected by centrifugation at 1000 × g for 10 min at room temperature. The combined supernatants were acidified with 4 N HCl to pH 1.5 and phenolic acids were processed as mentioned in the case of free phenolic acids and it was designated as *Andrographis serpyllifolia* Bound Phenolic Fraction (ASBP). Extraction was performed in triplicate to evaluate the yield with statistical significance.

### *Determination of total phenolics*

The concentration of phenol content in the extracted free and bound phenolic fractions of AS - ASFP and ASBP were determined according to Folin - Ciocalteu method[16] and results were expressed as gallic acid equivalents. Samples (0.1 mL) were mixed with 1 mL of 2 fold diluted Folin - Ciocalteu reagent and 2 mL of 10% sodium carbonate solution. The absorbance was measured at A765 nm with Shimadzu UV-visible spectrophotometer after incubating for 30 min at room temperature.

### *HPLC analysis of phenolics of ASFP and ASBP*

Phenolic acids in ASFP and ASBP were analyzed using RP-HPLC (model LC-10A, Shimadzu, Shimadzu Corp. Tokyo, Japan) employing Phenomenex $C_{18}$ column (250 × 4.6 mm) using a diode array UV-detector operating at $\lambda$ max A280 nm. A solvent system consisting of water/acetic acid/methanol (80:5:15, v/v/v) was used as mobile phase at a flow rate of 1mL/min. Phenolic acids in phenolic fractions were identified and quantified by matching the retention time and peak area against those of standards under similar experimental conditions.

### *Antiproliferative activity of ASFP and ASBP*

Human cervical cancer (HeLa) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Cells were grown in DMEM supplemented with 1% penicillin-streptomycin, 2 mM L-glutamine and foetal bovine serum (10%), and, the cells were incubated at 37 °C in 5 % $CO_2$ atmosphere with the media refreshed every 2 days as previously reported.[7]

Antiproliferative activity was determined in the presence and absence of ASFP and ASBP using MTT assay.[17] Aliquots of $2 \times 10^5$ exponentially growing HeLa cells were seeded on 96-well flat microtitre plates. After 4 h of incubation at 37 °C in 5% $CO_2$, the growth medium was replaced by media containing different concentrations of ASFP and ASBP; the plates were incubated for 48 h again. MTT solution (5 mg/mL in phosphate buffer saline - PBS, filter sterilized) were added to each well and incubated for further 4 h at 37 °C. The formazan product thus formed was solubilized by the addition of 100 μL of DMSO and optical density was read at 570 nm. Cell proliferation (percent) was expressed as percentage of viable cells of treated to control. At least three replications for each sample were used to determine the effect of ASFP and ASBP on cell proliferation. $IC_{50}$, the concentration under which a 50% inhibition of cell proliferation observed was calculated.

## Assessment of oxidant and antioxidant status

### *Measurement of cellular malondialdehyde*

Malondialdehyde was measured according to the method of Karatas et al[18] to estimate the extent of cellular stress. An aliquot (100 μL) of cell suspension; both control and treated were mixed 250 μL of perchloric acid and 650 μL of distilled water and incubated for 30 min at 37 °C. The samples were centrifuged at 4500 × g for 5 min and subjected to HPLC. HPLC was carried out on $C_{18}$ column using solvent system of 30 mM $KH_2PO_4$: Methanol (65:35 v/v) with a flow rate of 1 mL/min and fractions were monitored at 254 nm. MDA concentration was quantitated using TMP (1, 1, 3, 3-tetramethoxy propane) as standard.

### *Measurement of glutathione*

Estimation of the glutathione was done according to Phenomenex protocol provided by manufacturer (Phenomenex Ltd., USA). An aliquot of cell suspensions - both treated and controlled cells were mixed with 50 mM phosphoric acid containing 0.1 mM EDTA, centrifuged at 3000 × g for 10 min 4 °C and the supernatant was subjected to HPLC. HPLC was carried out on Synergi Hydro $C_{18}$ (4.5 × 250 mm, Phenomenex Ltd., USA) using a solvent system of 20 mM potassium phosphate pH 2.7: Acetonitrile (99:1 v/v) with a flow rate of 1 mL/min. Fractions were monitored automatically at A215 nm and glutathione concentration was quantitated using glutathione standard.

## Assessment of antioxidant activity in ASFP and ASBP in vitro

### *Inhibition of lipid peroxidation*

The formation of thiobarbituric acid reactive substances (TBARS) was used to monitor lipid peroxidation in rat liver homogenate.[19] A 10 % fresh rat liver homogenate was prepared in 10 mM Tris-HCl buffer containing 175 mM KCl, pH 7.4. Briefly 0.5 mL of liver homogenate was preincubated with ASFP and ASBP for 5 min at 37 °C. The peroxidation was initiated by the addition of 10 μM $Fe^{2+}$/100 μM ascorbate by incubating at 37 °C for 1 h. The reaction was terminated by the addition of TCA-TBA-HCl solution (15% trichloroacetic acid; 0.375% TBA; 0.25 N HCl) together with 0.02% BHT. The solution was heated in a boiling water bath for 15 min and cooled. The precipitate was removed by centrifugation at 2500 × g for 10 min at room temperature and TBARS in the supernatant was determined at 532 nm. The ratio percent inhibition was calculated from the following equation.

$$\%\ \text{Inhibition} = \left[\frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}}\right] \times 100$$

### *Inhibition of protein carbonyls*

Protein carbonyls were measured spectrophotometrically as previously described.[20] Briefly, 0.8 mL of rat liver homogenate was incubated at 37 °C in 10 mM Tris-HCl buffer containing 175 mM KCl, pH 7.4. ASFP and ASBP were added at concentration of 2-10 μg GAE/mL before initiation of oxidation by 10 μM Ferrous sulfate/100 μM ascorbate. After incubation for 1h, 0.2 mL of 10 mM DNPH (dissolved in 2 N HCl) was added, accompanied by blanks with 0.2 mL of 2 N HCl alone. Following the incubation, proteins were precipitated with an equal volume of 20% trichloroacetic acid on ice for 10 min and washed three times with 1.5 mL of an ethanol/ethyl acetate mixture (1:1v/v ). Finally the precipitate was dissolved in 3% SDS in Sodium phosphate buffer (pH 6.8) at 37 °C with ultrasonic vibration for 15 min and the absorbance was measured at A360 nm.

### *DNA protection ability*

DNA protection assay was performed as previously described.[21] Briefly, calf thymus DNA (1 μg) was added to Fenton's reagent (30 mM $H_2O_2$, 50 μM ascorbic acid and 80 μM $FeCl_3$) containing 2-4 and 8-16 μg of ASFP and ASBP respectively. The final volume of the mixture was brought up to 20 μL, incubated for 30 min at 37 °C and, the DNA was analyzed on 1% agarose gel followed by ethidium bromide staining. Fragmented DNA moves faster on the gel and the effect of ASFP and ASBP against DNA damage was evaluated.

### *Free radical scavenging activity using DPPH*

The effect of free and bound phenolics of AS on DPPH radical was measured according to the method of Lai et al.[22] Aliquots of 100 μL of free and bound phenolic fractions (1-5 μg GAE/mL) and standard antioxidants were mixed with 100 mM Tris - HCl buffer (800 μL, pH 7.4) and then added 1 mL of 500 μM DPPH in methanol (final concentration of 250 μM), the mixture was shaken vigorously and allowed to stand for 20 min at room temperature in dark. Changes in absorbance of the samples were measured at A517 nm. Free radical scavenging activity (FRS) was expressed as the inhibition percentage and was calculated using the following formula.

$$\text{FRS } (\%) = \left[\frac{A_{\text{Control (517nm)}} - A_{\text{Sample (517nm)}}}{A_{\text{Control (517nm)}}}\right] \times 100$$

### *Reducing power activity*

The reducing power of free and bound phenolics was determined according to the method of Yen and Chen.[23] Aliquots of free and bound phenolic extracts (5-25 μg GAE/mL) were added to equal volume of 0.2 M phosphate buffer, pH 6.6 and 1 % potassium ferricyanide and mixture was incubated at 50 °C for 20 min. Equal volume of 10% TCA was added to the mixture, which was then centrifuged at 600 × g for 10 min at room temperature The supernatant was mixed with distilled water and 0.1 % ferric chloride at a ratio of 1:1:2 (v/v/v) and then absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

### *Statistical Analysis*

All the experiments were carried out in triplicates (n = 3) and the results were expressed as mean ± standard deviation (SD). The correlation coefficients between total phenolics and the antioxidant activity were demonstrated by employing SPSS (version 10.0.2) at a level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

### *Total phenolic content (Free and Bound phenolics)*

Phenolic compounds are widely distributed in the plant kingdom and are particularly attractive as prophylactic agents due to their high prevalence in the diet and also due to their pharmacological effects.[24] Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through reactions involving their carboxylic and hydroxyl groups, respectively. This allows phenolic acids to form cross-links with cell wall macromolecules.[25,26] Hence in the present study, we isolated free (ASFP) and bound (ASBP) phenolic fractions from *A. serpyllifolia*. The total phenolic content in ASFP and ASBP fractions was found to be 15.1 ± 0.2 mg GAE/g and 0.6 ± 0.09 mg GAE/g respectively. Approximately 25 fold higher yields of phenolics was noticed in ASFP fraction as measured by the Folin-Ciocalteau method.

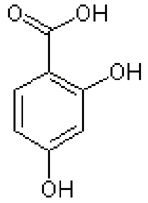
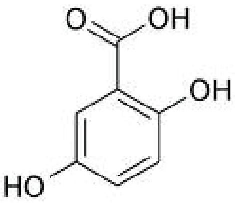
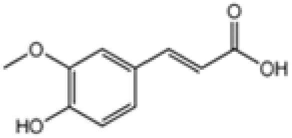
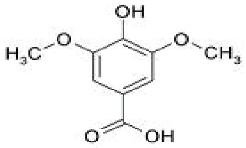
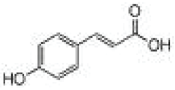
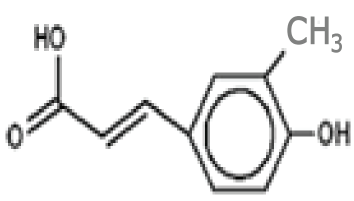
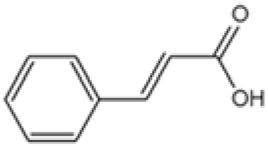
### *HPLC analysis of free and bound phenolics*

Nature of phenolics present in free and bound phenolic fractions were found to be responsible for their potency, hence they were analyzed on HPLC and nature of phenolic acids (mg per gram) present in ASFP and ASBP given in Table 1 and the chromatograms in Figure 1. Andrographolide reported to be the major component of AS was absent in the both ASFP and ASBP fractions. ASFP, which exhibited stronger antioxidant activities than ASBP, was found to contain higher amounts of gentisic, syringic, caffeic and ferulic acids that are more potent as free radical scavengers with $IC_{50}$ of 1.9, 1.8, 3.0 and 4.6 μg/mL respectively that may contribute to the activity. In ASBP however, less potent phenolic acids – *p*-coumaric ($IC_{50}$~64.9 μg/mL) and cinnamic acid ($IC_{50}$ ~ 81 μg/mL) were present in abundant amounts (~88 %). Hence antioxidant potency of ASFP was 9 fold higher than ASBP.

### *Antiproliferative activity*

In order to ascertain whether ASFP and ASBP fractions contribute to anticancer properties, effects of ASFP and ASBP fractions on human cervical adenocarcinoma cell lines were studied. Treatment of HeLa cells with ASFP and ASBP showed a dose dependent growth inhibition. ASFP and ASBP also suppressed cell proliferation as evidenced by MTT assay together with morphological changes. Morphologically cells looked irregular, shrunken and disrupted. The highest dose (20 μg/mL) of ASFP resulted in ~80 % inhibition of cell growth after 24 h of treatment. When treated with ASBP (20 μg/mL), only 24% inhibition of cell growth was found. Comparing the $IC_{50}$ value of antiproliferative activity, it can be found that ASFP ($IC_{50}$ – 12.5 μg/mL) showed stronger antiproliferative activity than ASBP ($IC_{50}$ – 41.6 μg/mL). (Table 2). Consequently, the difference in the phenolic content appears to contribute to different degree antiproliferative properties. Present experimental data also suggest that components within the ASFP may have inherent properties that suppress cancer cell proliferation

**Table 1.** The antioxidant potency and phenolic content of ASFP and ASBP

| Phenolic compounds | General structures | Antioxidant activity $IC_{50}$ (μg) | Phenolic Content mg/g | |
|---|---|---|---|---|
| | | | ASFP | ASBP |
| **Protocatechuic acid**<br>(3,4-Dihydroxybenzoic acid) |  | 1.35 ± 0.1$^{a}$ | 3.1 | – |
| **Gentisic acid**<br>(2,5-Dihydroxy benzoic acid) |  | 1.9 ± 0.1$^{a}$ | 9.1 | 0.15 |
| **Caffeic acid**<br>(3,4-Dihydroxy cinnamic acid ) |  | 3.0 ± 0.2$^{b}$ | 2.3 | – |
| **Syringic acid**<br>(4-Hydroxy-3,5 dimethoxy benzoic acid) |  | 1.8 ± 0.1$^{b}$ | 0.18 | 0.04 |
| ***p*-coumaric acid**<br>(p-Hydroxy cinnamic acid**)** |  | 64.9 ± 1$^{a}$ | – | 0.31 |
| **Ferulic acid**<br>(4-hydroxy-3-methoxy cinnamic acid) |  | 4.6 ± 0.2$^{a}$ | 18.7 | 0.15 |
| **Cinnamic acid**<br>(3-Phenyl-2-propenoic acid) |  | 81 ± 1.2$^{a}$ | 0.025 | 5.07 |

ASFP and ASBP containing different phenolic acids given with their yield mg/g and structure. Comparative $IC_{50}$ (concentration of sample or standard required to scavenge 50% of the DPPH free radicals) values of ASFP, ASBP compared with standard antioxidants. $^{a}p < 0.001$, $^{b}p < 0.01$ $^{c}p < 0.05$. Samples were analyzed statistically employing Origin 6.1 SPSS (version 10 for windows XP, SPSS Inc.).

*Antioxidant activity measured as free radical scavenging activity

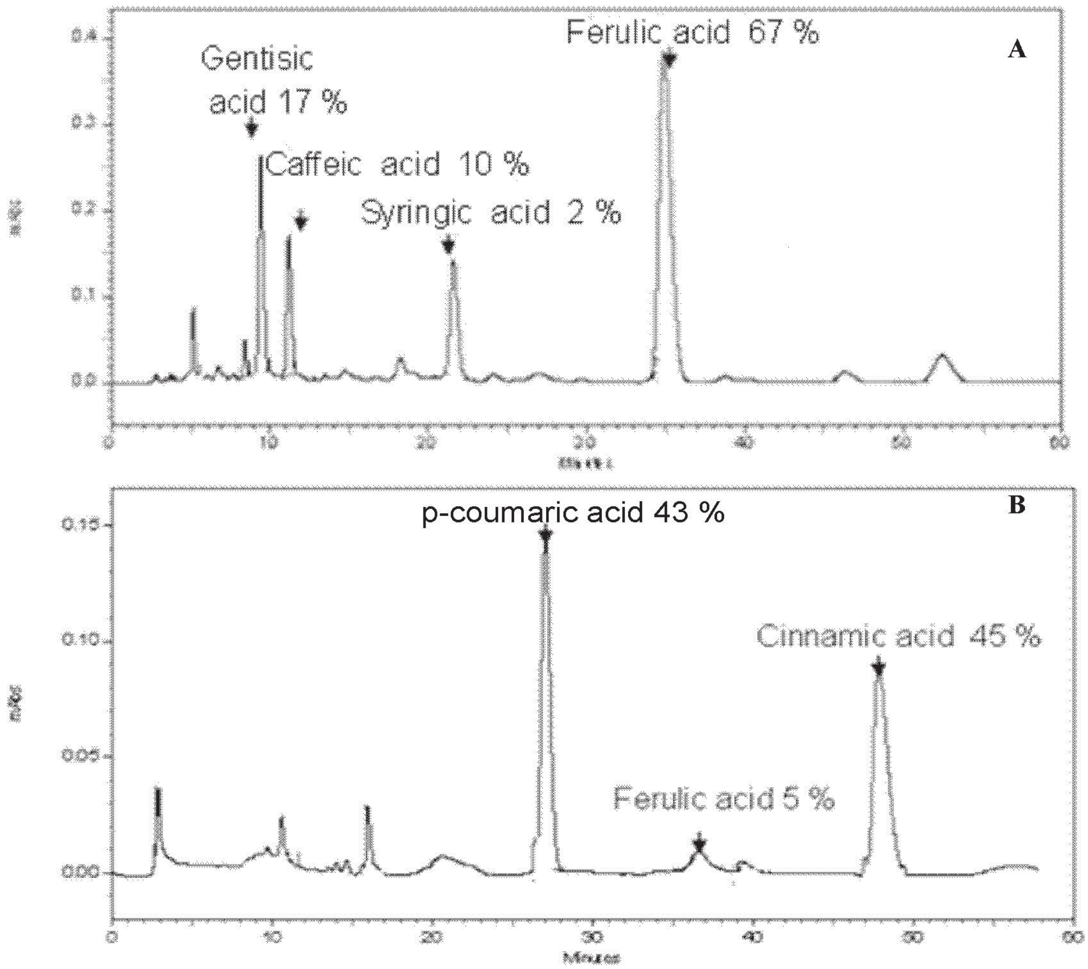


**Figure 1.** HPLC analysis of phenolic acid constituents in free (ASFP) and bound (ASBP) phenolic fractions. *Phenolic acids in ASFP (A) and ASBP (B) fraction were identified by comparison of their retention time with known standards.*

**Table 2.** Inhibition of proliferation, levels of glutathione and malondialdehyde in human cervical cancer (HeLa) cell lines upon treatment with ASFP and ASBP

| Cells | Inhibition of proliferation ($IC_{50}$ – μg/mL) | Glutathione nmoles/mg protein | MDA nmoles/mg protein |
|---|---|---|---|
| Control | – | $13.0 \pm 0.7^{a}$ | $1.3 \pm 0.2^{a}$ |
| ASFP treated | $12.5 \pm 0.7^{b}$ | $5.7 \pm 0.5^{a}$ | $4.2 \pm 0.4^{b}$ |
| ASBP treated | $41.6 \pm 1.5^{b}$ | $11.6 \pm 0.8^{b}$ | $1.5 \pm 0.2^{a}$ |

$^{a}p < 0.001$, $^{b}p < 0.01$ $^{c}p < 0.05$. Samples were analyzed statistically employing Origin 6.1 SPSS (version 10 for windows XP, SPSS Inc.).

## Measurement of cellular malondialdehyde and Glutathione

The incubation of HeLa cells with 20 μg GAE/mL of ASFP and ASBP for 24 h decreased cell viability along with a significant increase in lipid peroxidation (Table 2) as evidenced by accumulation of MDA, a marker of fatty acid oxidation. Approximately 3 and 1.2 fold increase in MDA content was observed for ASFP and ASBP respectively at 20 μg GAE/mL concentration. Moreover phenolic compounds can induce oxidative stress in cancer cells and hence the effect seen may be due to induction of such stress.[27-29] These data are consistent with the hypothesis that lipid peroxidation may be a potential mechanism for the inhibition of tumor cell growth.[30-33] Both ASFP and ASBP reduced the GSH levels in HeLa cells, but the extent of decrease was greater with ASFP than with ASBP (Table 2). Increase in MDA and decrease in GSH levels could be due to the induction of apoptosis as reported from other sources.[34,35]

The depletion of GSH may be one of the initial cellular responses to stress that induces apoptosis.[36] Therefore, artificial manipulation of intracellular GSH levels may be expected to enhance apoptosis. Many GSH based therapeutic strategies have focused on lowering GSH levels in order to increase sensitivity of cells to ionizing radiation, and to decrease the resistance to many chemotherapeutic drugs.[37] The detailed mechanisms by which ASFP and ASBP deplete GSH in these cells are as yet unknown. However studies have shown that GSH depletion can be seen to enhance the antitumor and cytotoxicity of various drugs without increasing toxicity to normal tissue.[38] A severe GSH depletion leaves cells more vulnerable to oxidative damage and is normally associated with calcium homeostasis disruption, which ultimately causes cell death.[39]

### Assessment of antioxidant capacity

Since ROS have been linked to cancer and several other chronic diseases because of their ability to cause oxidative protein carbonization, generation of reactive aldehydes (MDA) derived from lipid and lipoprotein per oxidation[40] and damage to DNA. Five independent and highly reproducible assays including inhibition of lipid peroxidation, protein carbonyls, protection to DNA, free radical scavenging and reducing power activity were employed for the evaluation of antioxidant capacities of the phenolic fractions.

### Inhibition of Lipid peroxidation

Combination of $Fe^{2+}$ and a reducing agent is an extensively used system for generating hydroxyl radicals to induce lipid peroxidation. As shown in Figure 2A, ASFP and ASBP inhibited TBARS formation significantly at almost all doses, indicating its value as a protector of lipid peroxidation through its interaction with radical species. At 10 μg GAE/mL concentration of ASFP and ASBP, the extent of inhibition was upto 82% and 18% respectively. ASFP showed maximum inhibition of lipid peroxidation with an $IC_{50}$ of 6 ± 0.4 μg GAE/mL compared to that of ASBP (27.7 ± 0.36 μg GAE/mL).

### Inhibition of protein oxidation

The oxidative protein damage was assessed by the formation of carbonyl compounds which may be an early marker for protein oxidation.[20] Figure 2B depicts the protective effect of ASFP and ASBP on protein oxidation. At 10 μg GAE concentration, ASFP prevented the protein oxidation by 68%, being the most active with an $IC_{50}$ of 7.3 ± 0.2 μg GAE/mL, when compared to that of ASBP, which was less effective, and showed only 22% inhibition with an $IC_{50}$ of 22.7 ± 0.3 μg GAE/mL.

### DNA protection ability

The increased electrophoretic mobility of DNA due to DNA fragmentation caused by Fenton's reagent was normalized upon treatment with ASFP and ASBP extracts prior to oxidative stress. A dose-dependent protection was observed by both ASFP and ASBP of AS at 2-4 and 8-16 μg GAE, respectively (Figure. 2C). Greater than 80% protection to native DNA during oxidation was observed by ASFP and ASBP at 2 and 16 μg GAE concentration respectively. These results indicate that free and bound phenolics of ASFP and ASBP can quench free radicals and thereby may protect the DNA against oxidation induced damage.

### Free radical scavenging activity

Both ASFP and ASBP showed concentration dependent radical scavenging activity (Figure. 2D). The free radical scavenging activity of ASFP was found to be higher compared to that of ASBP with $IC_{50}$ of 2.1 and 19.1 μg/mL GAE respectively. In order to understand the probable ability of individual phenolic acids present in extracts in scavenging free radical, pure phenolic acids were examined for their ability to scavenge DPPH radical. As indicated in Table 1, cinnamic acid and *p*-coumaric acid showed least activity with an $IC_{50}$ of 81 and 64.9 μg/mL respectively. The lower free radical scavenging potential of ASBP may be attributed to the presence of cinnamic and *p*-coumaric acid, which have least free radical scavenging potential.

### Reducing power activity

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[23] Therefore, the reductive capabilities in terms of $Fe^{3+}$ – $Fe^{2+}$ transformation were measured in the presence of ASFP and ASBP. Figure. 2E indicates a dose-dependent

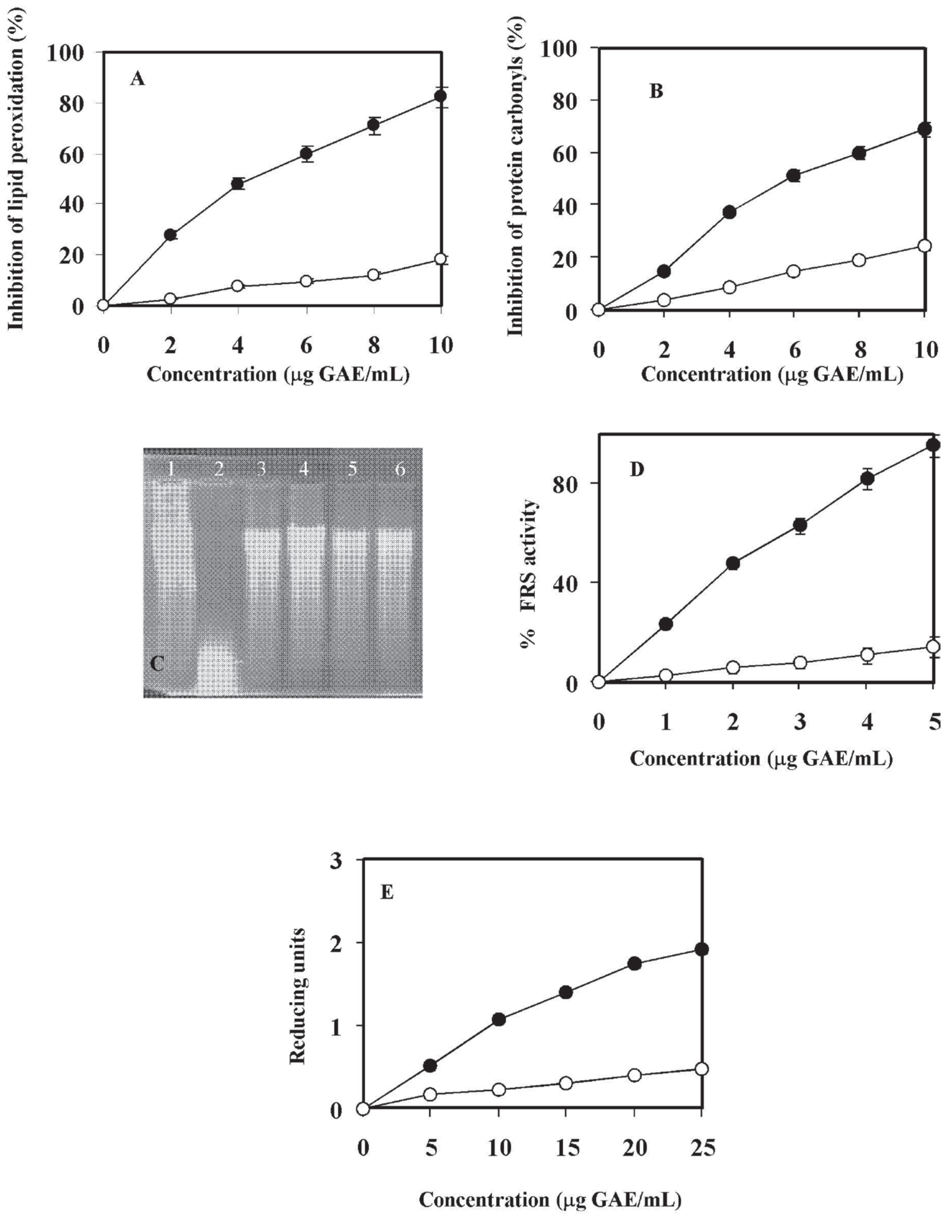


**Figure 2.** Antioxidant potency of ASFP (-●-) and ASBP (-○-).
*Inhibition of TBARS formation,* **(B)** *Inhibition of Protein carbonyls*, **(C)** *DNA protection ability*: *1 μg of native calf thymus DNA in (Lane 1); DNA treated with Fenton's reagent (Lane 2); DNA pretreated with 2-4 μg of ASFP (Lane 2, 3) and ASBP (Lane 3, 4).* **(D)** *DPPH scavenging activity and* **(E)** *Reducing power activity of ASFP (-●-) and ASBP (-○-).*

increase in the activity of ASFP and ASBP. Approximately five-folds increase in activity was observed in ASFP over that of ASBP, which correlates well with the total phenolic concentration in ASFP (correlation coefficient $R^2 = 0.9684$, $p = 0.002$). This difference could be due to the constituent phenolic acids present in each.

Results suggest thus that ASFP and ASBP can quench/scavenge free radicals and thereby may protect the biomolecules against oxidation induced damage. The multi-potent antioxidative mechanism of ASFP and ASBP suggest the counteracting effect of ROS mediated cellular or molecular damages during cancer.

In order to understand the role of phenolic acids in contributing to antioxidant activity in various assays, antioxidant potency was compared between ASFP and ASBP and correlation coefficient between phenolics and the activity was calculated. Results indicated that there is a correlation between reducing power activity, DNA protection, and inhibition of lipid peroxidation, protein carbonyls and antiproliferative activity with phenolics as indicated by correlation coefficient varying from 0.96 to 1.0 with an average r value of 0.98. In addition to higher phenolics in ASFP, FRS activity was higher (9 fold) in ASFP, which could be attributed to the differences in phenolic acid composition with different antioxidant potency. Maximum activity of 43% followed by 24% and 19% was contributed by gentisic acid, ferulic acid, and protocatechuic acid in ASFP and gentisic (39%), cinnamic (30%) and ferulic (16%) acids in ASBP respectively.

## CONCLUSION

Humans are exposed to a wide variety of naturally occurring or artificial carcinogens and co carcinogens; many of which exert deleterious effects through the generation of ROS.[40] Thus, removal of excess ROS or suppression of their generation is an efficient way of preventing cancer. Therefore, dietary or medicinal intake of antioxidants could be an important regimen for fortifying the body's defence system against oxygen free radical-induced carcinogenesis. The daily ingestion of antioxidant nutrients or phytochemicals can scavenge oxygen free radicals that may be generated in respiratory processes and/or xenobiotic metabolism, thereby mitigating the oxidative stress in our body. Current paper emphasizes the potential role of multi-potent antioxidative source - *A. serpyllifolia* to employ as a source for exogenous protection against cellular/molecular damages during disease condition. Separation of free and bound phenolic fractions followed by demonstration of differential antioxidant activity of phenolic acid composition in them enabled us to correlate the key role and involvement of phenolic acids against various steps of ROS mediated cancer pathogenecity. Further the study also substantiated the major contribution of phenolic acids against several steps of cancer pathogenesis as opposed to the less abundant molecule reported earlier - andrographolide, serpyllin etc. Most plant polyphenols possess both antioxidant as well as prooxidant properties[35] and prooxidant action of polyphenolics may be an important mechanism of their anticancer and apoptosis inducing-properties.[34] This may be the mechanism for observed antioxidant and antiproliferative property of phenolic fractions of *A. serpyllifolia*. The positive correlation between phenolic content of *A. serpyllifolia* to its antioxidant and antiproliferative activity supports the above observation. The content and nature of phenolic acids in the phenolic fractions therefore may explain their antioxidant and antiproliferative abilities to be responsible for killing cancer cells. Potent antiproliferative and DNA protective activity of ASFP and ASBP may also contribute significantly against cancer pathogenesis.

In conclusion, all of these results show that the *A. serpyllifolia* extracts can be used as an easily accessible source of natural antioxidants against oxidative stress induced cancer and other chronic diseases. The results of antioxidant and antiproliferative activities together with our earlier report on antimetastatic potency of *A. serpyllifolia* may provide support for some of the uses *of A. serpyllifolia* in folk medicine against cancer.

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