Evaluation of antimutagenic and protective effects of *Parkinsonia aculeata* L. leaves against H₂O₂ induced damage in pBR322 DNA

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Abstract The in vitro antimutagenic and DNA protecting potential of organic (methanol, hexane, n-butanol) and aqueous extract/fractions of *Parkinsonia aculeata* L. (Fabaceae) was investigated by employing Ames assay and DNA nicking assay. DNA damage by hydroxyl radicals was effectively inhibited by all the extract/fractions. A marked antimutagenic effect was observed against 4-Nitro-*o*-phenylenediamine and sodium azide (direct acting mutagens) and 2-Aminofluorene (indirect acting mutagen) in TA98 and TA100 strains of *Salmonella typhimurium*. In Ames assay, two different modes of experiments i.e. pre-incubation and co-incubation were performed and it was observed that all the extract/fractions showed better results in the pre-incubation as compared to co-incubation mode. Out of all the extract/fractions tested, n-butanol fraction was found to be the most effective in preventing DNA damage and inhibiting mutagenesis. UHPLC analysis of extract/fractions revealed presence of polyphenols such as gallic acid, catechin, chlorogenic acid, caffeic acid, umbelliferone, coumaric acid, rutin, and ellagic acid etc. DNA protecting and antimutagenic activity of this plant could be attributed to presence of these polyphenols. The results of this study indicate the presence of potent antioxidant factors in *Parkinsonia aculeata* L., which are being explored further for their mechanism of action.

Keywords DNA nicking · Antimutagenic · *Salmonella typhimurium* · UHPLC

Introduction

The genotoxic chemicals present in the environment, as a result of pollution and industrialization cause various diseases like cancer, by causing alternation in genetic makeup. These genotoxic chemicals react with oxygen and nitrogenous bases leading to the production of free radicals and dimers that cause disability in living organisms (Ramachandran and Prasad 2008). Cancer is a disease in which a single normal body cell undergoes genetic transformation into a cancer cell and begins to reproduce as in uncontrollable number of cells (Varmus and Weinberg 1993; Devi et al. 2015). There is a correlation between the carcinogenicity and mutagenicity as mutagens leads to proliferation and progression of cancer (Ogunjobi et al. 2007; Kundu et al. 2009; Tan and Spivack 2009). Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide or hydroxyl radicals cause oxidative stress in living cells which results in cell ageing (Drew and Leeuwenburgh 2002), neurodegenerative diseases (Liebert et al. 2006; Zhao and Zhao 2013), DNA damage (Orrenius et al. 2007) and cancer (Hadjiladis 1997; Huang 2003) etc. Antioxidant compounds can inhibit the oxidation of compounds at different stages, either by breaking radical-chains or by chelating catalyst metals.

Since, the oxidants and mutagens are involved in the initiation and promotion of several disorders in living beings, so the significance of phytochemicals in counteracting the mutagenic or carcinogenic effects is gaining interest. Antimutagens are the chemicals that reduce the mutagenic effects of mutagens or carcinogens. Now days, antimutagenic

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Flowchart 1 Extraction procedure of leaves of *Parkinsonia aculeata* L

properties of various plant secondary metabolites and dietary constituents have been reported (Ames 1983; Aqil et al. 2008; Azuma et al. 2011). The antimutagenic and DNA protecting nature of plant products has been attributed due to the presence of many phytochemicals like flavonoids, phenols etc. (Negi et al. 2003; Shon et al. 2004; Ramos et al. 2011; Jaberian et al. 2013). Secondary metabolites are the promising source of antioxidants/antimutagens present in plants. It has been reported that a decrease in the rate of oxidants or mutations would decrease the probability of occurrence of cancer like diseases and might be adequate in the lifetime of living beings (Loeb et al. 2003; Rajaei et al. 2010). Now a day the interest in the plant based drug has increased due to advancement in technological approaches used in designing and preparations of drugs (Nobili et al. 2009; Boivin et al. 2009; Yasukawa et al. 2009). Although, a number of plants, enriched with phytochemical compounds, have been explored for their antimutagenic and DNA protecting properties, yet many plants have still not been adequately evaluated. So, considering all the facts, the present study was focused to investigate the antimutagenic and hydroxyl radical scavenging potential of leaves of *Parkinsonia aculeata* L. (*P. aculeata*), against direct and S9 dependent mutagens in TA98 and TA100 strains of *Salmonella typhimurium*.

P. aculeata L. is a perennial flowering tree from the family Fabaceae. It is widely cultivated in South America, Mexico, South to North Argentina and now introduced and cultivated in South Africa, Uganda and India also

Fig. 1 DNA nicking assay for Methanol extract of *Parkinsonia aculeata* L. leaves. (DNA Deoxyribose degradation assay, DW Distilled water, FR Fenton's reagent)

(Wagner et al. 1999; Pier 2000; Divya et al. 2011; Sharma and Vig 2013). Leaves of this plant are prescribed for malaria, fever and as an abortifacient. Leaves have been reported to contain various c-glycosylflavones like vitexin, iso-vitexin, orientin, iso-orientin and Parkintin etc. (Bhatia et al. 1966; Ali et al. 2005). To the best of our knowledge there is no study available in the literature reporting on the antimutagenic and DNA protecting nature of these extract/fractions of *P. aculeata* leaves. Therefore, this investigation aimed to identify the properties and presence of polyphenolic compounds in extract/fractions, so as to facilitate their use in phytomedicines.

Materials and methods

Chemicals

All chemical reagents (sodium chloride, histidine, biotin, glucose, calcium chloride, magnesium sulphate, ammonium chloride, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, agar, luria broth, etc.) and solvents (methanol, n-butanol, hexane and DMSO) were of analytical grade. 4 Nitro-o-phenylenediamine (NPD), 2-aminoflourene (2AF), Nicotinamide adenine dinucleotide phosphate (NADP), Glucose-6-Phosphate, sodium azide,

Table 1 Densitometric analysis of effect of Methanol extract of *Parkinsonia aculeata* L. leaves on different forms of DNA

	Control	FR	Rutin	200 μg/ml	400 μg/ml	600 μg/ml	800 μg/ml	1000 μg/ml
Form I DNA	75.211	19.178	69.796	90.133	91.726	93.022	89.829	94.76
Form II DNA	24.789	65.934	30.204	09.867	08.274	06.978	10.171	05.24
Form III DNA	0	14.888	0	0	0	0	0	0
Total DNA	100	100	100	100	100	100	100	100

Fig. 2 DNA nicking assay for Hexane fraction of *Parkinsonia aculeata* L. leaves. (DNA Deoxyribose degradation assay, DW Distilled water, FR Fenton's reagent)

tris buffer, EDTA, boric acid, ethidium bromide, bromophenol blue, agarose, pBR322 DNA, hydrogen peroxide, ferric chloride and ascorbic acid were obtained from Sigma Chemical Co. and Hi-Media, Mumbai, India. TA98 and TA100 tester strains were procured from Institute of Microbial Technology (CSIR), Chandigarh, India.

Plant material and extraction procedure

The leaves of plant were collected from the region of Chheharta, Amritsar, India. Botanical identification of the plant was made from the Herbarium of Department of Botanical and Environmental Sciences, GNDU, and a set of sample (Accession No. 6774) was deposited in the herbarium of the department. Leaves were thoroughly washed with water, dried and ground to very fine powder followed by extraction with different solvents using maceration method (Flowchart 1). The supernatants obtained of different extract/fractions were filtered and concentrated using rotary evaporator (Buchi Rotavapor) to obtain respective extract/fractions.

Ultra high performance liquid chromatography (UHPLC)

All the extract/fractions of leaves were subjected to UHPLC in order to identify the presence of various polyphenolic compounds like gallic acid, epicatechin, umbelliferone, coumaric

acid, and so forth. The preparation of sample to identify active constituents is as mentioned below.

Sample preparation

For UHPLC analysis, the dried extract/fractions were dissolved in methanol (HPLC grade) and filtered through 0.22 µm syringe filter. Plant samples were analyzed on Shimadzu UHPLC Nexera system (Schimadzu, USA) with photodiode array detector. C18 column (150 mm × 4.6 mm) was used at 25 °C temperature. Mobile phase used for analysis was consisted of solution I (0.1 % acetic acid) and solution II (methanol). Flowrate was 1 mL/min and injection volume was 5 µL. Content of peaks was determined using software provided with Shimadzu UHPLC Nexera system (USA).

DNA nicking assay

DNA nicking assay was performed according to the method given by Lee et al. (2002). Supercoiled pBR322 plasmid DNA (0.3 µl) and 10 µM Fenton's reagent (50 µM ascorbic acid, 80 µM FeCl₃ and 30 mM H₂O₂) were mixed with, different concentrations of extract/fractions. The reaction mixture was incubated at 37 °C for 30 min and the final volume of the reaction mixture was raised up to 20 µl with distilled water. Rutin was used as a positive control whereas in negative control, Fenton's reagent was replaced with same volume of dH₂O. The reaction mixture was then incubated for 30 min at 37 °C. 2.5 µl of bromophenol blue was added in each sample followed by electrophoresis in TBE buffer and DNA bands were then analyzed on gel documentation system (Gel Doc XR, Bio-Rad, USA).

Densitometric analysis of DNA cleavage

The amount of different forms of DNAs i.e. Form I (supercoiled), Form II (single standard nicked and open circular) and Form III (double standard nicked and linear) was calculated by using Quantity one[®] software version 4.5.2 of BIORAD. The protective effect of extract/fractions was measured by quantifying the amount of different nicked forms of DNA as compared to negative control.

Table 2 Densitometric analysis of effect of Hexane fraction of *Parkinsonia aculeata* L. leaves on different forms of DNA

	Control	FR	Rutin	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
Form I DNA	96.516	0	84.694	18.927	24.322	34.514	30.206	47.594
Form II DNA	3.484	96.654	15.306	81.073	75.678	65.486	62.021	52.155
Form III DNA	0	3.346	0	0	0	0	7.773	0.251
Total DNA	100	100	100	100	100	100	100	100

Fig. 3 DNA nicking assay for n-Butanol fraction of *Parkinsonia aculeata* L. leaves. (DNA Deoxyribose degradation assay, DW Distilled water, FR Fenton's reagent)

Antimutagenic activity

The antimutagenicity of different extract/fractions of *P. aculeata* L. was determined on TA98 and TA100 strains of *Salmonella typhimurium* using *Salmonella* histidine point mutation assay. This method was proposed by Maron and Ames (1983) and modified by Grover and Bala (1993). Direct acting mutagens used for TA98 and TA100 strains were 4 Nitro-o-phenylenediamine (NPD) and sodium azide (SA). All the extract/fractions and mutagens were prepared in DMSO, except SA that was dissolved in distilled water (dH₂O). The concentrations of extract/fractions used for evaluating the antimutagenicity was 50, 100, 250, 500, 1000, 2500 and 3000 µg/0.1 ml/plate. Two methodologies, co-incubation and pre- incubation were followed in order to characterize antimutagenicity of compounds.

Bacterial culture was grown overnight in Luria broth (LB) liquid medium. In the co-incubation method, 100 µL each of bacterial culture, mutagen, and different concentrations of the extract/fractions was added to 2 mL of top agar. In the pre-incubation method, equal volumes of mutagen and extract were mixed and incubated for 30 min at 37 °C. Then after incubation, 200 µL of pre-incubated solution and 100 µL of bacterial culture was added to 2 mL of top agar. The top agar was then poured on minimal agar plates followed by incubation for 48 h at 37 °C. Each concentration was tested in triplicate. Spontaneous (100 µL

bacterial culture), positive control (100 µL bacterial culture and 100 µL mutagen) and negative control (100 µL bacterial culture and 100 µL extract/fractions) were also run with pre- and co- incubation.

Many chemical carcinogens are known to get activated in the presence of cytochrome-based P450 metabolic activation system (Phase I) and their highest activity is present in the liver of lower animals. In case of bacteria, an exogenous activation system is required to be added because, they have limited metabolic capability. For this purpose, to study the effect of plant extract/fractions on the mutagenicity of 2AF (indirect-acting mutagen), post-mitochondrial supernatant (S9 fraction) from rat liver homogenate was used.

Preparation of S9 fraction

The rat liver homogenate was prepared by the method given by Garner et al. (1972). 0.1 % phenobarbitol was given to male albino rats (150 to 200 g weight), in drinking water for 6 days. On the seventh day, the rats were sacrificed and livers were excised out. The excised livers were weighed and washed several times with chilled sterilized 0.15 mol/L KCl solutions to ensure blood free preparation of sample. The washed livers were minced and homogenized, and centrifuged for 10 min at 9000 rpm. The supernatant was separated in the flask and distributed in 2 ml cryovials and stored in refrigerator for one night and then transferred to liquid nitrogen till needed. S9 mix was prepared on the day of experiment by adding 16.75 mL of distilled water, 25 mL of sodium phosphate buffer (pH 7.4), 2 mL of NADP, 0.25 mL of G – 6-P, 1 mL of MgCl₂-KCl salt solution, and 5 mL of rat liver S9. S9 mix was maintained at chilled condition while performing the experiment. S9 mix (500 µL), bacterial culture (100 µL), and mutagen (100 µL) were mixed with 2 mL top agar and poured onto the minimal plates. After 48 h of incubation, the number of revertant his⁺ bacterial colonies were scored. The different concentrations of extract/fractions were considered to be non-toxic only if the number and size of bacterial colonies in negative control was equivalent to that of spontaneous colonies. The inhibitory activity of each test sample was

Table 3 Densitometric analysis of effect of n-Butanol fraction of *Parkinsonia aculeata* L. leaves on different forms of DNA

	Control	FR	Rutin	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
Form I DNA	83.324	0	64.064	78.305	66.532	83.225	89.698	91.513
Form II DNA	16.676	65.986	35.936	21.695	31.692	16.775	10.302	8.487
Form III DNA	0	34.014	0	0	1.776	0	0	0
Total DNA	100	100	100	100	100	100	100	100

Fig. 4 DNA nicking assay for Aqueous fraction of *Parkinsonia aculeata* L. leaves. (DNA Deoxyribose degradation assay, DW Distilled water, FR Fenton's reagent)

expressed as percent decrease of reverse mutations as follows:

$$\text{Inhibition(\%)} = (x-y)/(x-z) \times 100$$

Where, 'x' means number of histidine revertants induced by mutagen, 'y' means number of his⁺ revertants induced by mutagen in the presence of extract and 'z' denotes the number of revertants induced in absence of mutagen.

Statistical analysis

Each plant extract/fractions were tested using triplicate plates per experiment. The data is presented as the mean and the standard error of experiment with triplicate plates/dose. The data was analyzed using analysis of variance (One way and Two Way ANOVA) for statistical significance. High range statistical domain (HSD) using Tukey's test was used to compare the difference among the means of different modes of treatment and positive control.

Results and discussion

Considerable attention has been focused in recent years on the exploration of medicinal plants for the prevention of diseases caused by free radicals and mutagens (Greenwald 1996). Reactive Oxygen Species (ROS) are continuously generated

in the biological system as the byproduct of various metabolic activities. ROS like hydroxyl radicals act as initiators of carcinogenesis and mutagenesis as they are able to cause damage to cellular biomolecules such as proteins, nucleic acid and various membrane components (Halliwell and Gutteridge 1985; Ardestani and Yazdanparast 2007; Singh et al. 2009). To withstand this problem the potential antioxidant substances like phenols, flavonoids etc. are tested for their antioxidative/anticarcinogenic activities (Arouma 2003). Therefore, this is the first report where we study the DNA protecting and antimutagenic activity of different extract/fractions of the leaves of *P. aculeata* L using DNA nicking assay and Ames assay.

DNA nicking assay

The chemopreventive agents exert their effect on DNA replication and repair processes, to modulate the cellular mutagenic processes (Mitic-Culafic et al. 2009). DNA nicking assay is employed to check the ability of leaves extract/fractions of *P. aculeata*, against the DNA damage caused by free radicals. Breakage in DNA strands occurs, when plasmid DNA comes in the exposure of Fenton's reaction (FR) and generates radicals which react with nitrogenous bases of DNA and leads to the production of sugar and base radicals. These base radicals then react with sugar, and results in breakage of sugar-phosphate backbone. DNA nicking converts helical DNA into linear or relaxed form (Form II and Form III).

The addition of plant extract/fractions to the reaction mixture, prevent the breaking of DNA strand by OH radicals and transformation of supercoiled DNA (Form I) to Form II and III DNA. The plasmid DNA treated with FR and different concentrations of MEH extract produce strong Form I DNA, which confirmed the scavenging ability of MEH (Table 1 and Fig. 1). At the minimum (200 μg/ml) and maximum (1000 μg/ml) concentrations, MEH showed 90.13 % and 94.76 % of supercoiled DNA (Form I). As shown in Fig. 2 and Table 2, it was observed that hexane (HEX) fraction show low effect against OH radicals. At the highest concentration i.e. 1000 μg/ml, 47.594 % of Form I DNA of pBR322 plasmid was found which is very low as compared to rutin. All the

Table 4 Densitometric analysis of effect of Aqueous fraction of *Parkinsonia aculeata* L. leaves on different forms of DNA

	Control	FR	Rutin	200 μg/ml	400 μg/ml	600 μg/ml	800 μg/ml	1000 μg/ml
Form I DNA	73.079	0	66.089	60.587	50.219	65.608	42.234	49.151
Form II DNA	26.921	78.654	33.911	39.413	49.781	34.392	55.737	47.966
Form III DNA	0	21.346	0	0	0	0	2.029	2.883
Total DNA	100	100	100	100	100	100	100	100

Table 5 *In vivo* antimutagenic activity of 80 % Methanol extract of *Parkinsonia aculeata* L. leaves against mutagenicity of direct and promutagens in TA98 and TA100 strains of *Salmonella typhimurium*

Treatments	Dose ($\mu\text{g}/0.1 \text{ ml}$)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE
Spontaneous		33.29 $\pm$ 1.96	–	37.36 $\pm$ 1.87	–	196.3 $\pm$ 4.32	–	202 $\pm$ 34.7	–
Positive control									
NPD	20	1078.0 $\pm$ 51.29	–		–	–	–	–	–
Sodium azide	25	–	–		–	2379.8 $\pm$ 27.29	–	–	–
2-AF	20	–	–	3498.9 $\pm$ 152.2	–	–	–	2994 $\pm$ 121.6	–
Negative control									
	50	33.26 $\pm$ 1.67	–	37.26 $\pm$ 1.78	–	196.1 $\pm$ 1.23	–	202.4 $\pm$ 23.8	–
	100	33.27 $\pm$ 1.34	–	37.34 $\pm$ 1.43	–	196.6 $\pm$ 1.67	–	201.8 $\pm$ 34.5	–
	250	33.24 $\pm$ 1.78	–	37.29 $\pm$ 2.13	–	196.3 $\pm$ 2.31	–	201.8 $\pm$ 21.5	–
	500	33.28 $\pm$ 1.56	–	37.36 $\pm$ 1.70	–	196.4 $\pm$ 1.23	–	202.1 $\pm$ 24.7	–
	1000	33.29 $\pm$ 2.56	–	37.34 $\pm$ 1.27	–	196.2 $\pm$ 2.31	–	202.3 $\pm$ 18.9	–
	2000	33.30 $\pm$ 2.34	–	37.33 $\pm$ 2.12	–	196.7 $\pm$ 1.22	–	202.1 $\pm$ 34.8	–
	3000	33.26 $\pm$ 2.67	–	37.35 $\pm$ 2.56	–	196.2 $\pm$ 2.31	–	202.3 $\pm$ 24.6	–
Co-incubation									
	50	1067 $\pm$ 5.548	0.97 $\pm$ 0.531 ^a	3476.8 $\pm$ 12.49	0.63 $\pm$ 0.359 ^a	2234.5 $\pm$ 18.77	6.65 $\pm$ 0.860 ^a	2867 $\pm$ 582.8	4.54 $\pm$ 0.201 ^a
	100	1053.1 $\pm$ 12.03	2.38 $\pm$ 0.641 ^{ab}	3321.7 $\pm$ 17.02	5.12 $\pm$ 0.492 ^{ab}	2156.3 $\pm$ 20.03	10.24 $\pm$ 0.918 ^a	2487 $\pm$ 78.12	18.15 $\pm$ 6.349 ^{ab}
	250	1002.2 $\pm$ 19.38	7.25 $\pm$ 1.828 ^b	3167.8 $\pm$ 53.11	9.56 $\pm$ 1.542 ^{bc}	2098.4 $\pm$ 33.34	12.88 $\pm$ 1.512 ^a	2239 $\pm$ 99.65	27.03 $\pm$ 3.569 ^b
	500	934.4 $\pm$ 9.713	13.74 $\pm$ 0.930 ^c	2989.9 $\pm$ 70.36	14.70 $\pm$ 2.033 ^{cd}	1894.4 $\pm$ 50.6	22.23 $\pm$ 2.317 ^b	1256 $\pm$ 84.1	62.25 $\pm$ 3.01 ^c
	1000	894.6 $\pm$ 5.196	17.55 $\pm$ 0.494 ^c	2803.9 $\pm$ 26.33	20.07 $\pm$ 0.761 ^{de}	1802.3 $\pm$ 2.00	26.45 $\pm$ 0.092 ^{bc}	1004 $\pm$ 9.86	71.28 $\pm$ 0.375 ^{cd}
	2000	837.3 $\pm$ 11.26	23.04 $\pm$ 1.059 ^d	2678.1 $\pm$ 32.32	23.71 $\pm$ 0.934 ^{ef}	1733.5 $\pm$ 38.21	29.60 $\pm$ 1.744 ^c	884 $\pm$ 43.25	75.57 $\pm$ 1.549 ^{cd}
	3000	746.8 $\pm$ 10.84	31.70 $\pm$ 1.038 ^e	2467.9 $\pm$ 89.52	29.78 $\pm$ 2.586 ^f	1543.5 $\pm$ 4.333	38.29 $\pm$ 0.199 ^d	678 $\pm$ 88.32	82.96 $\pm$ 6.607 ^d
HSD		5.013		7.075		6.409		15.779	
F-ratio _(6,14)		115.302*		50.927*		75.079*		93.774*	
Pre-incubation									
	50	1057.8 $\pm$ 14.27	1.93 $\pm$ 0.330 ^a	3356.4 $\pm$ 78.69	4.12 $\pm$ 2.284 ^a	2168.4 $\pm$ 100	9.68 $\pm$ 4.758 ^a	2234 $\pm$ 126.6	27.22 $\pm$ 4.537 ^a
	100	1006.9 $\pm$ 5.239	6.80 $\pm$ 0.565 ^b	3216.7 $\pm$ 61.19	8.15 $\pm$ 1.768 ^a	1982.7 $\pm$ 64.49	18.18 $\pm$ 2.954 ^{ab}	1989 $\pm$ 124.2	35.99 $\pm$ 4.448 ^a
	250	987.9 $\pm$ 1.764	8.62 $\pm$ 0.178 ^b	2879.9 $\pm$ 52.94	17.88 $\pm$ 1.530 ^b	1734.4 $\pm$ 132.3	29.55 $\pm$ 6.057 ^{abc}	1456 $\pm$ 64.1	55.08 $\pm$ 2.275 ^b
	500	879.8 $\pm$ 3.18	18.97 $\pm$ 0.331 ^c	2432.8 $\pm$ 109.9	30.79 $\pm$ 3.009 ^c	1539.0 $\pm$ 124.5	38.50 $\pm$ 5.700 ^{bcd}	1109 $\pm$ 102	67.51 $\pm$ 3.652 ^{bc}
	1000	832.8 $\pm$ 12.2	23.47 $\pm$ 1.167 ^d	1976.9 $\pm$ 12.24	43.96 $\pm$ 0.354 ^d	1345.4 $\pm$ 179.6	47.37 $\pm$ 8.223 ^{cd}	984 $\pm$ 111	71.99 $\pm$ 3.977 ^{cd}
	2000	789.6 $\pm$ 3.28	27.60 $\pm$ 0.314 ^e	1534.2 $\pm$ 12.51	56.76 $\pm$ 2.076 ^e	1093.5 $\pm$ 58.94	58.92 $\pm$ 2.700 ^d	824 $\pm$ 37.21	77.72 $\pm$ 2.766 ^{cd}
	3000	745.8 $\pm$ 0.882	31.79 $\pm$ 0.139 ^f	1292.4 $\pm$ 18.81	63.74 $\pm$ 0.540 ^e	984.6 $\pm$ 120.4	63.89 $\pm$ 5.515 ^d	619 $\pm$ 120.7	85.07 $\pm$ 1.511 ^d
HSD		2.614		9.029		26.193		16.794	
F-ratio _(6,14)		443.044*		157.426*		14.009*		39.417*	

Table 5 (continued)

Treatments	Dose ($\mu\text{g}/0.1\text{ ml}$)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE
Two independent-Way ANOVA									
Treatment		F-ratio _(1,28) : 51.972*		F-ratio _(1,28) : 371.972*		F-ratio _(1,28) : 65.615*		F-ratio _(1,28) : 38.820*	
Dose		F-ratio _(6,28) : 375.003*		F-ratio _(6,28) : 203.173*		F-ratio _(6,28) : 32.124*		F-ratio _(6,28) : 124.096*	
Treatment x Dose		F-ratio _(6,28) : 3.919*		F-ratio _(6,28) : 30.655*		F-ratio _(6,28) : 2.794*		F-ratio _(6,28) : 5.709*	
HSD:		4.244		8.703		20.461		17.485	

Bold Figures shows percentage inhibition of extract/fractions and HSD and F-ratio

NPD 4-nitro-o-phenylenediamine, *AF* 2-aminofluorene; *represents the significance at $p \leq 0.05$; Data shown are Mean $\pm$ SE of an experiment performed in triplicate; %age inhibition followed by same letters are not significant different using HSD Multiple comparison test

concentrations of fraction showed high amount of single standard DNA i.e. Form II DNA as comparatively to Form I DNA i.e. supercoiled form.

n-butanol (BUT) fraction of *P. aculeata* L. showed effective DNA protecting potential against the damage caused by OH radical (Fig. 3 and Table 3). It was found that pBR322 DNA, in the presence of FR alone, has high amount of Form II DNA (65.986 %) as compared to Form III DNA (34.014 %). Even at the low concentration i.e. 200 $\mu\text{g}/\text{ml}$, BUT fraction showed high amount of Form I DNA (78.305 %) as compared to rutin (64.064 %). It was clear from the Fig. 4 and Table 4, that aqueous (AQU) fraction showed very less potential to preserve the supercoiled form of pBR322 DNA. At the highest concentration of AQU fraction i.e. 1000 $\mu\text{g}/\text{ml}$, 49.151 % of Form I DNA was preserved. Rutin preserved 66.089 % of Form I DNA which is higher than the potential of AQU fraction. In the presence of FR alone, 78.654 % and 21.346 % amount of Form II DNA and Form III DNA was calculated. Form III DNA i.e. double standard nicked linear DNA was also observed at the higher concentrations of AQU fraction. Among all the extract/fractions of *P. aculeata* L. leaves, BUT fraction showed maximum hydroxyl scavenging effect followed by MEH, AQU and HEX fractions.

As very scanty information is available on the biological properties of *P. aculeata* plant, so no such DNA protecting nature of this plant is reported in the literature. But many researchers reported the inhibitory effect of plant extract on plasmid DNA nicking caused by hydroxyl radicals (Kumar and Chattopadhyay 2007; Kowsalya et al. 2013; Golla and Bhimathati 2014 and Leba et al. 2014). All the extract/fractions of plant inhibit the effect of hydroxyl radicals produced by FR. Butanol fraction showed maximum DNA protection whereas hexane fraction showed minimum effect against FR.

Antimutagenic activity

Several researchers have used the *Salmonella typhimurium* assay to examine the antimutagenic activity of plants used in traditional medicines (Horn and Vargas 2003; Reid et al. 2006; Verschaeve and van Staden 2008; Zahin et al. 2010; Unlu et al. 2010; Tsai et al. 2012). Hung et al. 2009 classified mutagenesis inhibitors into three categories on the basis of their modes of action viz. co-incubation, pre-incubation and blocking agents. Co-incubation method is used to determine the bio-antimutagenic effect of phytochemicals. These phytochemicals suppress the effect of mutagens by either directly inactivating the mutagens or by suppressing the activity of metabolic enzymes. Pre-incubation method is used to evaluate the desmutagenic effect and third category involves antimutagens that exert their effect by modifying the function of bacterial cells in order to reduce the DNA mutations and act as blocking agent.

Table 6 *In vivo* antimutagenic activity of hexane fraction of *Parkinsonia aculeata* L. leaves against mutagenicity of direct and promutagens in TA98 and TA100 strains of *Salmonella typhimurium*

Treatments	Dose ($\mu\text{g}/0.1\text{ ml}$)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE
Spontaneous		33.64 $\pm$ 1.46	–	41.46 $\pm$ 1.33	–	197.6 $\pm$ 17.89	–	192.3 $\pm$ 13.2	–
Positive control									
NPD	20	1379 $\pm$ 12.86	–	–	–	–	–	–	–
Sodium azide	25	–	–	–	–	1678 $\pm$ 108.2	–	–	–
2-AF	20	–	–	4983.1 $\pm$ 63.53	–	–	–	3426.5 $\pm$ 137.7	–
Negative control									
	50	33.61 $\pm$ 2.75	–	41.43 $\pm$ 3.45	–	197.3 $\pm$ 7.89	–	191.4 $\pm$ 11.4	–
	100	33.64 $\pm$ 2.35	–	41.48 $\pm$ 3.65	–	196.9 $\pm$ 8.98	–	191.8 $\pm$ 17.2	–
	250	33.62 $\pm$ 1.38	–	41.44 $\pm$ 2.32	–	197.2 $\pm$ 9.84	–	192.3 $\pm$ 12.3	–
	500	33.64 $\pm$ 2.85	–	41.44 $\pm$ 3.78	–	197.4 $\pm$ 12.7	–	192.1 $\pm$ 12.5	–
	1000	33.63 $\pm$ 1.89	–	41.45 $\pm$ 2.45	–	197.3 $\pm$ 14.8	–	192.4 $\pm$ 13.5	–
	2000	33.62 $\pm$ 2.43	–	41.46 $\pm$ 1.78	–	197.7 $\pm$ 13.7	–	192.2 $\pm$ 12.6	–
	3000	33.61 $\pm$ 2.89	–	41.47 $\pm$ 1.88	–	197.6 $\pm$ 12.7	–	192.4 $\pm$ 15.2	–
Co-incubation									
	50	1368.5 $\pm$ 5.659	0.78 $\pm$ 0.400^a	4976.7 $\pm$ 102.5	0.23 $\pm$ 0.108^a	1669.9 $\pm$ 73.74	0.54 $\pm$ 0.274^a	3412.1 $\pm$ 123.8	0.44 $\pm$ 0.138^a
	100	1364.6 $\pm$ 5.965	1.07 $\pm$ 0.399^a	4789.5 $\pm$ 175.3	3.92 $\pm$ 1.535^{ab}	1661.5 $\pm$ 93.05	1.11 $\pm$ 0.455^a	3387.3 $\pm$ 181.5	1.21 $\pm$ 0.366^a
	250	1354.3 $\pm$ 7.572	1.83 $\pm$ 0.812^{ac}	4345.8 $\pm$ 39.0	12.89 $\pm$ 0.789^{bc}	1589.3 $\pm$ 46.56	5.99 $\pm$ 3.144^{ab}	3202.6 $\pm$ 202.7	6.92 $\pm$ 3.883^a
	500	1349.6 $\pm$ 3.333	2.18 $\pm$ 0.248^{ac}	4323.9 $\pm$ 220.4	13.34 $\pm$ 4.460^{bc}	1574.5 $\pm$ 19.17	6.99 $\pm$ 1.295^{ab}	2898.3 $\pm$ 59.83	16.33 $\pm$ 1.837^b
	1000	1327.6 $\pm$ 17.32	3.82 $\pm$ 1.288^{ac}	4209.5 $\pm$ 122.0	15.65 $\pm$ 2.470^c	1504.4 $\pm$ 31.75	11.72 $\pm$ 2.144^b	2678.3 $\pm$ 10.53	23.13 $\pm$ 0.325^b
	2000	1323.5 $\pm$ 15.34	4.12 $\pm$ 1.140^{ac}	4180.4 $\pm$ 77.95	16.24 $\pm$ 1.516^c	1496.7 $\pm$ 33.41	12.25 $\pm$ 2.300^b	2331.6 $\pm$ 49.01	33.85 $\pm$ 1.515^c
	3000	1309.6 $\pm$ 2.333	5.15 $\pm$ 0.173^{bc}	3987.7 $\pm$ 12.02	20.14 $\pm$ 0.243^c	1467.8 $\pm$ 21.3	14.19 $\pm$ 1.374^b	1989.3 $\pm$ 15.39	44.43 $\pm$ 0.475^d
HSD		3.664		10.219		8.872		8.418	
F-ratio _(6,14)		5.093[*]		11.212[*]		8.768[*]		92.892[*]	
Pre-incubation									
	50	1329.1 $\pm$ 3.61	3.71 $\pm$ 0.268^a	4924.5 $\pm$ 442.2	1.20 $\pm$ 0.406^a	1587.7 $\pm$ 30.94	6.09 $\pm$ 2.071^a	3219.9 $\pm$ 132.0	6.42 $\pm$ 2.351^a
	100	1318.6 $\pm$ 15.72	4.48 $\pm$ 1.178^a	4796.3 $\pm$ 34.93	3.78 $\pm$ 0.707^{ab}	1573.6 $\pm$ 23.10	7.04 $\pm$ 1.559^a	2985.4 $\pm$ 32.12	13.63 $\pm$ 1.012^a
	250	1306.1 $\pm$ 13.65	5.42 $\pm$ 1.014^a	4532.6 $\pm$ 117.5	9.12 $\pm$ 2.096^{ab}	1543.4 $\pm$ 14.29	9.08 $\pm$ 0.965^{ab}	2434.3 $\pm$ 144.7	30.67 $\pm$ 4.475^b
	500	1301.3 $\pm$ 20.53	5.77 $\pm$ 1.526^a	4509.4 $\pm$ 83.76	9.58 $\pm$ 1.695^{ab}	1521.9 $\pm$ 4.33	10.54 $\pm$ 0.293^{ab}	1982.3 $\pm$ 42.40	44.65 $\pm$ 1.311^c
	1000	1289.6 $\pm$ 21.09	6.64 $\pm$ 1.567^a	4486.3 $\pm$ 51.39	10.05 $\pm$ 1.040^b	1509.0 $\pm$ 7.572	11.41 $\pm$ 0.511^{ab}	1543.4 $\pm$ 27.24	58.22 $\pm$ 0.844^d
	2000	1273.3 $\pm$ 7.02	7.85 $\pm$ 0.522^a	4402.8 $\pm$ 24.37	11.74 $\pm$ 0.493^b	1486.1 $\pm$ 17.84	12.96 $\pm$ 1.205^{bc}	1256.9 $\pm$ 14.50	67.08 $\pm$ 0.448^{de}
	3000	1256.1 $\pm$ 54.25	9.13 $\pm$ 1.803^a	3606.8 $\pm$ 166.9	27.85 $\pm$ 3.376^c	1423.7 $\pm$ 10.00	17.18 $\pm$ 0.654^c	1189.6 $\pm$ 54.88	69.16 $\pm$ 1.697^e
HSD		5.992		8.546		5.738		10.413	
F-ratio _(6,14)		2.302		23.307[*]		10.058[*]		137.757[*]	

Table 6 (continued)

Treatments	Dose (μg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE
Two independent-Way ANOVA									
Treatment		F-ratio _(1,28) : 39.624*		F-ratio _(1,28) : 1.458		F-ratio _(1,28) : 13.883*		F-ratio _(1,28) : 501.628*	
Dose		F-ratio _(6,28) : 6.027*		F-ratio _(6,28) : 29.596*		F-ratio _(6,28) : 17.168*		F-ratio _(6,28) : 226.836*	
Treatment x Dose		F-ratio _(6,28) : 0.096		F-ratio _(6,28) : 2.785*		F-ratio _(6,28) : 1.128		F-ratio _(6,28) : 14.961*	
HSD		5.329		10.108		8.017		10.117	

Bold Figures shows percentage inhibition of extract/fractions and HSD and F-ratio

NPD 4nitro-o-phenylenediamine, *AF* 2-aminofluorene; *represents the significance at $p \leq 0.05$; Data shown are Mean $\pm$ SE of an experiment performed in triplicate; %age inhibition followed by same letters are not significant different using HSD Multiple comparison test

In Ames assay, it was found that all the extract/fractions of *P. aculeata* L. were non-toxic to TA98 and TA100 strains of *Salmonella typhimurium* at all the concentrations (50 $\mu\text{g}/0.1\text{ ml}/\text{plate}$ to 3000 $\mu\text{g}/0.1\text{ ml}/\text{plate}$) tested and inhibited mutagenicity in a dose-dependent manner. Non toxicity of extract/fractions against the bacteria was checked by negative control (only extract) as a well-developed lawn with almost similar number of colonies as that of spontaneous were observed. A dose dependent relationship was found between concentrations of extract/fractions and percentage of antimutagenic activity. All extract/fractions were found to be effective in inhibiting the mutagenicity of S9 dependent mutagens (2-AF) and direct acting mutagens (NPD in TA98 and sodium azide in TA100 strain).

High percentage inhibition was found in pre-incubation mode of experiment as compared to co-incubation in all the test samples. Table 5 shows that methanol (MEH) extract of leaves exhibited 31.70 % and 31.79 % inhibitory activity at the maximum dose tested (3000 $\mu\text{g}/0.1\text{ ml}$) in co-incubation and pre-incubation mode of treatment, respectively against NPD mutagen in TA98 strain of *S. typhimurium*. In TA100 strain the effect of mutagenicity induced by sodium azide is reduced by 38.29 % and 63.89 % in co-incubation and pre-incubation mode of treatment. These results showed that MEH exhibit similar effects against NPD in co-incubation and pre-incubation modes of treatment even at the maximum dose tested. Table 5 also indicates the efficacy of MEH to prevent the 2-AF induced mutations in TA98 strain of *S. typhimurium* with percentage inhibition of 29.78 % and 63.74 % in co- and pre- incubation. However, in TA100 strain, the extract exhibited percentage inhibition of 82.96 % and 85.07 % in co- and pre- incubation modes of treatment.

Hexane fraction (HEX) was found to be ineffective in preventing mutagenicity, induced by NPD and sodium azide in TA98 and TA100 strains of *S. typhimurium* in both the modes of treatment (Table 6). HEX exhibited inhibitory activity of 5.15 % and 9.13 % in co-incubation and pre-incubation mode of treatment, at maximum concentration (3000 $\mu\text{g}/0.1\text{ ml}$) tested in TA98 strain. In TA100 strain, HEX exhibited percentage inhibition of 14.19 % and 17.18 % in co-incubation and pre-incubation mode of treatment, respectively (Table 6). Table 6 also showed the effect of HEX against the mutagenicity induced by 2-AF in TA100 by 44.43 % and 69.16 % inhibition in co- and pre- incubation mode of treatment which is comparatively higher then percentage inhibition i.e. 20.14 % and 27.85 % of TA98. From the results, it was found that HEX reduced the mutagenicity induced by S9 dependent mutagen in TA100 strain of *S. typhimurium* as compared to TA98 strain.

Butanol fraction (BUT) showed similar antimutagenic activity against NPD mutation in TA98 strain, in both co-incubation and pre-incubation mode of treatments with

Table 7 *In vivo* antimutagenic activity of n-butanol fraction of *Parkinsonia aculeata* L. leaves against mutagenicity of direct and promutagens in TA98 and TA100 strains of *Salmonella typhimurium*

Treatments	Dose ($\mu\text{g}/0.1\text{ ml}$)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE
Spontaneous		37.64 $\pm$ 1.46	–	39.86 $\pm$ 2.33	–	189.7 $\pm$ 8.56	–	202 $\pm$ 34.7	–
Positive control									
NPD	20	1089 $\pm$ 136.8	–	–	–	–	–	–	–
Sodium azide	25	–	–	–	–	2451 $\pm$ 123.9	–	–	–
2-AF	20	–	–	3988.5 $\pm$ 268.2	–	–	–	2994 $\pm$ 1.155	–
Negative control									
	50	37.59 $\pm$ 2.55	–	39.83 $\pm$ 2.78	–	189.6 $\pm$ 24.74	–	202.1 $\pm$ 23.8	–
	100	37.60 $\pm$ 2.76	–	39.86 $\pm$ 4.89	–	189.7 $\pm$ 23.45	–	201.6 $\pm$ 34.5	–
	250	37.62 $\pm$ 2.33	–	39.84 $\pm$ 3.46	–	189.3 $\pm$ 44.67	–	201.8 $\pm$ 21.5	–
	500	37.64 $\pm$ 2.55	–	39.82 $\pm$ 4.88	–	189.4 $\pm$ 38.80	–	202.2 $\pm$ 24.7	–
	1000	37.62 $\pm$ 2.98	–	39.85 $\pm$ 3.67	–	189.6 $\pm$ 24.90	–	202.1 $\pm$ 18.9	–
	2000	37.64 $\pm$ 2.33	–	39.84 $\pm$ 4.55	–	189.4 $\pm$ 24.97	–	202.2 $\pm$ 34.8	–
	3000	37.63 $\pm$ 2.66	–	39.86 $\pm$ 3.88	–	189.8 $\pm$ 14.82	–	201.9 $\pm$ 24.6	–
Co-incubation									
	50	989.6 $\pm$ 21.14	9.45 $\pm$ 2.01 ^a	3355.3 $\pm$ 88.09	16.03 $\pm$ 2.231 ^a	2432.2 $\pm$ 55.35	0.83 $\pm$ 0.116 ^a	2867 $\pm$ 13.49	4.53 $\pm$ 0.483 ^a
	100	976.5 $\pm$ 63.60	10.70 $\pm$ 6.027 ^a	2945.5 $\pm$ 398.3	26.41 $\pm$ 10.09 ^a	2356.7 $\pm$ 39.68	4.17 $\pm$ 1.299 ^a	2487.4 $\pm$ 156.2	18.14 $\pm$ 5.595 ^{ab}
	250	932.4 $\pm$ 51.75	14.89 $\pm$ 4.922 ^a	1487.7 $\pm$ 253	63.33 $\pm$ 6.407 ^b	2045.4 $\pm$ 72.8	17.93 $\pm$ 3.21 ^b	2239.5 $\pm$ 133.1	27.02 $\pm$ 4.769 ^b
	500	879.5 $\pm$ 29.42	19.93 $\pm$ 2.798 ^a	979.7 $\pm$ 12.37	75.19 $\pm$ 0.313 ^{bc}	1983.6 $\pm$ 0.674	20.67 $\pm$ 0.03 ^{bc}	1256.3 $\pm$ 211.9	62.24 $\pm$ 7.586 ^c
	1000	864.4 $\pm$ 35.0	21.36 $\pm$ 3.329 ^a	545.8 $\pm$ 25.12	87.16 $\pm$ 0.636 ^c	1745.5 $\pm$ 4.43	31.19 $\pm$ 0.197 ^{cd}	1004.4 $\pm$ 22.13	71.26 $\pm$ 0.793 ^{cd}
	2000	823.4 $\pm$ 45.16	25.26 $\pm$ 4.296 ^a	287.6 $\pm$ 57.5	93.72 $\pm$ 1.456 ^c	1632.4 $\pm$ 78.83	36.19 $\pm$ 3.637 ^{de}	884.7 $\pm$ 46.94	75.55 $\pm$ 1.681 ^{cd}
	3000	789.3 $\pm$ 40.56	28.50 $\pm$ 3.847 ^a	62.8 $\pm$ 4.976	98.41 $\pm$ 0.126 ^c	1488.9 $\pm$ 52.55	42.55 $\pm$ 2.854 ^e	678.8 $\pm$ 52.26	82.92 $\pm$ 1.872 ^d
HSD		19.726		22.390		10.553		19.897	
F-ratio _(6,14)		3.115 [*]		50.481 [*]		52.133 [*]		58.303 [*]	
Pre-incubation									
	50	962.3 $\pm$ 36.97	12.05 $\pm$ 3.516 ^a	3267.4 $\pm$ 155.6	18.26 $\pm$ 3.939 ^a	2344.5 $\pm$ 5.907	4.71 $\pm$ 0.261 ^a	2234.9 $\pm$ 9.148	27.27 $\pm$ 0.323 ^a
	100	922.4 $\pm$ 31.12	15.84 $\pm$ 2.96 ^a	2786.4 $\pm$ 206.9	30.44 $\pm$ 5.241 ^b	2168.1 $\pm$ 57.92	12.51 $\pm$ 2.561 ^{ab}	1989.5 $\pm$ 4.799	35.97 $\pm$ 0.344 ^a
	250	899.3 $\pm$ 44.59	18.04 $\pm$ 4.241 ^a	1347.8 $\pm$ 9.441	66.87 $\pm$ 0.246 ^c	1984.8 $\pm$ 4.557	20.61 $\pm$ 0.202 ^b	1456.6 $\pm$ 37.56	55.06 $\pm$ 1.345 ^b
	500	888.7 $\pm$ 15.42	19.05 $\pm$ 1.498 ^a	967.4 $\pm$ 21.81	76.51 $\pm$ 0.552 ^{cd}	1647.4 $\pm$ 64.38	35.53 $\pm$ 2.847 ^c	1109.5 $\pm$ 5.776	67.50 $\pm$ 0.207 ^c
	1000	826.6 $\pm$ 58.07	24.96 $\pm$ 5.523 ^a	535.8 $\pm$ 3.034	87.44 $\pm$ 0.077 ^{de}	1345.5 $\pm$ 51.03	48.88 $\pm$ 2.256 ^d	984.4 $\pm$ 57.79	71.98 $\pm$ 2.07 ^{cd}
	2000	799.6 $\pm$ 72.77	27.53 $\pm$ 6.922 ^a	202.5 $\pm$ 9.295	95.08 $\pm$ 0.72 ^e	1165.9 $\pm$ 43.65	56.82 $\pm$ 1.93 ^{de}	824.4 $\pm$ 114.9	77.71 $\pm$ 4.114 ^{de}
	3000	765.4 $\pm$ 44.87	30.78 $\pm$ 4.268 ^a	45.78 $\pm$ 4.662	99.85 $\pm$ 0.118 ^e	997.1 $\pm$ 5.854	64.29 $\pm$ 0.259 ^e	619.5 $\pm$ 73.1	85.04 $\pm$ 2.618 ^e
HSD		21.444		12.099		8.878		10.022	
F-ratio _(6,14)		2.309		162.946 [*]		155.684 [*]		108.833 [*]	

Table 7 (continued)

Treatments	Dose (μg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE	Mean ± SE	%age inh. ± SE
Two independent-Way ANOVA									
Treatment		F-ratio _(1,28) : 1.284		F-ratio _(1,28) : 0.929		F-ratio _(1,28) : 142.293*		F-ratio _(1,28) : 41.845*	
Dose		F-ratio _(6,28) : 5.264*		F-ratio _(6,28) : 151.989*		F-ratio _(6,28) : 182.453*		F-ratio _(6,28) : 131.024*	
Treatment x Dose		F-ratio _(6,28) : 0.093		F-ratio _(6,28) : 0.091		F-ratio _(6,28) : 7.641*		F-ratio _(6,28) : 6.033*	
HSD		22.109		19.309		10.465		16.904	

Bold Figures shows percentage inhibition of extract/fractions and HSD and F-ratio

NPD 4nitro-o-phenylenediamine, *AF*: 2-aminofluorene; *represents the significance at $p \leq 0.05$; Data shown are Mean $\pm$ SE of an experiment performed in triplicate; %age inhibition followed by same letters are not significant different using HSD Multiple comparison test

inhibitory percentage of 28.50 and 30.78 %, respectively at the maximum dose tested i.e. 3000 $\mu\text{g}/0.1\text{ ml}$ (Table 7). However, in TA100 strain, it exhibited an inhibitory activity of 64.29 % in pre mode of incubation as comparatively to 42.55 % in co-incubation at maximum dose. BUT fraction was also found to exhibit strong inhibitory activity against S9 dependent mutagen. It shows 99.85 % inhibition in pre-incubation mode of treatment in TA98 as compared to 98.41 % reduction in co-incubation treatment. The aqueous (AQU) fraction was also found to be effective in reducing frame shift mutation induced by NPD in TA98 strain (Table 8). AQU exhibit an inhibitory activity of 18.623 % which is higher than 5.799 % in pre- and co- incubation modes of treatments. It showed 6.251 % and 13.500 % inhibition in co- and pre- incubation against TA100 strains of *S. typhimurium*. AQU also showed antimutagenic effect against promutagen (2-AF) with percentage inhibition of 9.664 % and 37.753 % in pre- incubation mode of treatment of TA98 and TA100 strains of *S. typhimurium*.

On the basis of chromatogram obtained for all the samples (Sharma and Vig 2013 and 2014), it was concluded that extract/fractions of *P. aculeata* leaves contain gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, ellagic acid, etc. (Table 9). From the present study, it was found that n-butanol fraction and methanol extract of *P. aculeata* leaves were more effective than hexane and aqueous fractions. The results of one way and Two way ANOVA revealed the antimutagenic effect of *P. aculeata* against direct (NPD and sodium azide) and indirect acting mutagens (2-AF) were statistically significant at $p \leq 0.05$. These observations are in agreement with the earlier reports of Aqil et al. (2008); Kaur et al. (2010); Bhagavathy et al. (2011); Sarac and Sen (2014) that observed the antimutagenic effect of different plants against different mutagens. Leaves of *P. aculeata* plant have been reported to contain C-glycosylflavones like orientin, isorientin, vitexin and iso-vitexin (Bhatia et al. 1966; Besson et al. 1980; Marzouk et al. 2013; Al-Youssef and Hassan 2015).

Ali et al. (1999) found the effect of hexane and methanol extracts of *P. aculeata* L against various bacteria like *E. coli*, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella boydii*, *Staphylococcus aureus* etc. In our study, among all extracts, n-butanol fraction was found to be the most effective followed by methanol extract. Earlier reports suggested that plants contain phenolic compounds and non-toxic glycosides that can release phenolics, which are toxic to microbial pathogens. Therefore, these active compounds may be responsible for the antibacterial activity of the tested organisms (Kamba and Hassan 2001).

The present study was undertaken to study the DNA protecting and antimutagenic activity of different extract/

Table 8 *In vivo* antimutagenic activity of aqueous fraction of *Parkinsonia aculeata* L. leaves against mutagenicity of direct and promutagens in TA98 and TA100 strains of *Salmonella typhimurium*

Treatments	Dose ($\mu\text{g}/0.1\text{ ml}$)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE	Mean $\pm$ SE	%age inh. $\pm$ SE
Spontaneous		36.33 $\pm$ 1.453	–	38.67 $\pm$ 1.764	–	179.7 $\pm$ 3.48	–	209.3 $\pm$ 9.262	–
Positive control									
NPD	20	1025 $\pm$ 7.446	–	–	–	–	–	–	–
Sodium azide	25	–	–	–	–	2068 $\pm$ 51.68	–	–	–
2-AF	20	–	–	4198 $\pm$ 102.4	–	–	–	2908 $\pm$ 41.84	–
Negative control									
	50	35.67 $\pm$ 2.404	–	42.33 $\pm$ 2.028	–	178.7 $\pm$ 3.18	–	210.7 $\pm$ 2.404	–
	100	34.0 $\pm$ 1.155	–	37.67 $\pm$ 3.18	–	172.7 $\pm$ 5.783	–	208.3 $\pm$ 4.33	–
	250	35.0 $\pm$ 3.055	–	39.67 $\pm$ 4.372	–	171.3 $\pm$ 7.881	–	197.7 $\pm$ 2.028	–
	500	27.0 $\pm$ 2.082	–	30.33 $\pm$ 2.333	–	151.7 $\pm$ 13.97	–	195.3 $\pm$ 1.856	–
	1000	36.0 $\pm$ 1.155	–	28.33 $\pm$ 4.333	–	166.3 $\pm$ 15.24	–	195.0 $\pm$ 5.033	–
	2000	37.67 $\pm$ 3.756	–	28.67 $\pm$ 5.175	–	166.7 $\pm$ 10.73	–	196.3 $\pm$ 5.364	–
	3000	37.0 $\pm$ 2.646	–	38.62 $\pm$ 2.906	–	164.3 $\pm$ 6.386	–	198.3 $\pm$ 4.842	–
Co-incubation									
	50	1021 $\pm$ 2.887	0.400 $\pm$ 0.254 ^a	4059 $\pm$ 39.07	3.345 $\pm$ 0.94 ^a	2066 $\pm$ 0.577	0.106 $\pm$ 0.031 ^a	2825 $\pm$ 38.44	3.077 $\pm$ 1.425 ^a
	100	1018.6 $\pm$ 3.383	0.646 $\pm$ 0.341 ^a	4043 $\pm$ 30.73	3.726 $\pm$ 0.739 ^a	2037 $\pm$ 16.8	1.636 $\pm$ 0.887 ^{ab}	2695 $\pm$ 140.0	7.889 $\pm$ 5.186 ^a
	250	1005.6 $\pm$ 1.453	1.959 $\pm$ 0.147 ^{ab}	4040 $\pm$ 17.85	3.799 $\pm$ 0.429 ^a	2030 $\pm$ 16.26	2.003 $\pm$ 0.857 ^{ab}	2611 $\pm$ 65.9	10.958 $\pm$ 2.432 ^a
	500	1001 $\pm$ 6.928	2.405 $\pm$ 0.694 ^{ab}	4020 $\pm$ 26.76	4.271 $\pm$ 0.642 ^a	1992 $\pm$ 28.21	3.965 $\pm$ 1.472 ^{ab}	2493 $\pm$ 202.4	15.298 $\pm$ 7.46 ^a
	1000	992.7 $\pm$ 1.458	3.265 $\pm$ 0.845 ^{abc}	4013 $\pm$ 7.234	4.437 $\pm$ 0.173 ^{ab}	1968 $\pm$ 22.07	5.258 $\pm$ 1.16 ^b	2001 $\pm$ 272.6	33.432 $\pm$ 10.05 ^a
	2000	986.0 $\pm$ 1.528	3.950 $\pm$ 0.155 ^{bc}	3997 $\pm$ 44.95	4.821 $\pm$ 1.078 ^{ab}	1957 $\pm$ 25.53	5.838 $\pm$ 1.343 ^b	1974 $\pm$ 417.2	34.443 $\pm$ 15.38 ^a
	3000	967.7 $\pm$ 11.86	5.799 $\pm$ 1.201 ^c	3871 $\pm$ 26.51	7.862 $\pm$ 0.638 ^b	1949 $\pm$ 10.44	6.251 $\pm$ 0.548 ^b	1935 $\pm$ 45.9	35.908 $\pm$ 1.694 ^a
HSD		3.089		3.475		4.878		37.898	
F-ratio _(6,14)		8.819 [*]		4.448 [*]		5.478 [*]		3.191 [*]	
Pre-incubation									
	50	1000 $\pm$ 8.622	2.526 $\pm$ 0.871 ^a	4196.3 $\pm$ 30.01	0.041 $\pm$ 0.016 ^a	2048 $\pm$ 3.786	1.058 $\pm$ 0.2 ^a	2466 $\pm$ 170.5	16.387 $\pm$ 6.32 ^a
	100	989.3 $\pm$ 5.84	3.602 $\pm$ 0.589 ^a	4124 $\pm$ 63.4	1.778 $\pm$ 0.176 ^{ab}	1974 $\pm$ 30.11	4.959 $\pm$ 1.588 ^a	2118 $\pm$ 359.0	29.262 $\pm$ 13.3 ^a
	250	956.0 $\pm$ 4.619	6.969 $\pm$ 0.467 ^{ab}	4009 $\pm$ 12.58	4.546 $\pm$ 0.303 ^{abd}	1918 $\pm$ 15.87	7.908 $\pm$ 0.837 ^a	1994 $\pm$ 127.4	37.723 $\pm$ 4.702 ^a
	500	919.0 $\pm$ 8.145	10.621 $\pm$ 0.816 ^{bc}	3879 $\pm$ 19.06	7.654 $\pm$ 0.457 ^{bcd}	1894 $\pm$ 98.81	9.079 $\pm$ 5.156 ^a	1973 $\pm$ 258.5	34.467 $\pm$ 9.529 ^a
	1000	890.0 $\pm$ 5.568	13.650 $\pm$ 0.563 ^c	3871 $\pm$ 13.23	7.842 $\pm$ 0.312 ^{bcd}	1855 $\pm$ 162.3	11.200 $\pm$ 8.535 ^a	1956 $\pm$ 18.88	35.090 $\pm$ 0.696 ^a
	2000	880.3 $\pm$ 17.29	14.655 $\pm$ 1.751 ^{cd}	3669 $\pm$ 16.31	12.687 $\pm$ 3.913 ^{cd}	1849 $\pm$ 125.3	11.518 $\pm$ 6.59 ^a	1917 $\pm$ 23.26	36.545 $\pm$ 0.858 ^a
	3000	841.0 $\pm$ 9.866	18.623 $\pm$ 0.998 ^d	3796 $\pm$ 2.517	9.664 $\pm$ 0.06 ^d	1811 $\pm$ 67.55	13.500 $\pm$ 3.548 ^a	1885 $\pm$ 75.9	37.753 $\pm$ 2801 ^a
HSD		4.607		7.243		22.999		33.602	
F-ratio _(6,14)		39.509 [*]		8.836 [*]		0.808		1.113	

Table 8 (continued)

Treatments	Dose ($\mu\text{g}/0.1\text{ ml}$)	TA98	TA100
		Without S9	With S9
		Mean $\pm$ SE	Mean $\pm$ SE
		%age inh. $\pm$ SE	%age inh. $\pm$ SE
Two independent-Way ANOVA			
Treatment			
Dose		F-ratio _(1,28) : 295.373*	F-ratio _(1,28) : 7.031*
		F-ratio _(6,28) : 46.955*	F-ratio _(6,28) : 1.833
Treatment x Dose		F-ratio _(6,28) : 13.024*	F-ratio _(6,28) : 0.185
HSD		4.208	17.839
		6.095	38.432

Bold Figures shows percentage inhibition of extract/fractions and HSD and F-ratios

NPD 4-nitro-o-phenylenediamine, *AF* 2-aminofluorene; * represents the significance at $p \leq 0.05$; Data shown are Mean $\pm$ SE of an experiment performed in triplicate; %age inhibition followed by same letters are not significant different using HSD Multiple comparison test

Table 9 Chromatographic profile of polyphenolic compounds present in extract/fractions of *P. aculeata* L. leaves

S. No.	Polyphenolic compounds	Amount of compounds in Extract/Fractions (%age)			
		Methanol	Hexane	n-Butanol	Aqueous
1.	Gallic acid	0.006	–	0.005	0.001
2.	Catechin	0.015	0.011	0.045	0.022
3.	Chlorogenic acid	0.042	–	0.002	0.038
4.	Epicatechin	–	–	0.007	–
5.	Caffeic acid	0.047	0.002	0.212	0.020
6.	Umbelliferone	0.584	0.034	2.146	0.057
7.	Coumaric acid	0.063	0.004	0.215	0.005
8.	Rutin	0.061	–	0.020	0.037
9.	Ellagic acid	0.203	0.022	0.761	0.018
10.	Quercetin	0.145	0.005	–	–
11.	Kaempferol	0.018	0.024	–	–

Bold Figures shows percentage inhibition of extract/fractions and HSD and F-ratios

fractions of *P. aculeata* L. It was clear from the present study that leaves of *P. aculeata* L. exhibited significant DNA protecting property against FR and antimutagenic activity against promutagen and direct acting mutagens in TA98 and TA100 strains of *S. typhimurium* in a dose dependent manner.

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