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Full Length Research Paper

Antimutagenic potential of chickrassy (*Chukrasia tabularis* A. Juss.) bark

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The present study aims to assess the antimutagenic potential of methanol extract and different fractions (hexane, ethyl acetate and butanol) of chickrassy (*Chukrasia tabularis*), belonging to family meliaceae by employing histidine point reversion assay. The antimutagenic effect was evaluated against mutagens, 4-Nitro-*o*-phenylenediamine and sodium azide and promutagen, 2-Aminofluorene of TA98 and TA100 strain of *Salmonella typhimurium*. The co-incubation and pre-incubation mode of treatments were used to evaluate the bioantimutagenic and desmutagenic effects, respectively. From the results obtained, it was clear that methanol extract and its fractions showed more desmutagenic effect than bioantimutagenic effect. The methanol extract was found to be most active in TA98 while ethyl acetate fraction showed good results in TA100 strain against both promutagen and direct acting mutagen. High performance liquid chromatography (HPLC) analysis of methanol extract was carried out for the identification of chemical constituents and the results revealed that catechin, quercetin and rutin have contributed to its antimutagenic activity.

Key words: Chickrassy, bioantimutagen, desmutagen, *Salmonella typhimurium*, high performance liquid chromatography (HPLC).

INTRODUCTION

The chemicals and radiations, added to environment as a result of industrialization, impart cytotoxicity and anticipated different malformations and diseases in living organisms. These chemicals and radiations react with O_2 molecule - a basic molecule of aerobes and result in the production of free radicals such as $O_2^{-\cdot}$, $^{\cdot}OH$, H_2O_2 etc. Besides generating free radicals, the radiations and transition metals also interact directly with nitrogenous bases of deoxyribonucleic acid (DNA) molecule, leading to the production of dimers that ultimately affects genomic structure of living organisms (Elgazzar and Kazem, 2006; Ramachandran and Prasad, 2008). Among all the diseases, cancer is the most dreadful degenerative disease due to its multimechanistic and multifactorial nature and it is expected that there will be two-fold increase in the cancer related deaths in the next 50 years. In recent years, there is a paradigm shift in cancer control strategy from chemotherapy to chemoprevention that involves the usage of plant based drugs to combat the effect of mutagens either by detoxifying them at their entry to body by inducing phase II enzymes or may inactivate the reactive metabolites of mutagens that might be involved in the proliferation and progression of cancer and other degenerative diseases (Hong and Sporn, 1997; Kundu and Surh, 2009; Tan and Spivack, 2009). The use of antirisk factors to unfetter the organisms from the spitefull effects of hazardous chemicals that act as

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Abbreviations; 2-AF, 2-Aminofluorene; **NPD,** 4-nitro-o-phenylenediamine; **SA,** sodium azide; **DMSO,** dimethyl sulphoxide; **HPLC,** high performance liquid chromatography; **MEB,** methanol extract of bark; **HFB,** hexane fraction of bark; **EAFB,** ethyl acetate fraction of bark; **BFB**, butanol fraction of bark; **MNNG,** n-methyl-n'-nitro-n-nitrosoguanidine; **4NQO,** 4-nitroquinoline-*n*-oxide; **Trp-P-1,** 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole acetate; **B(α)P**: Benzo(α)Pyrene; **TFA,** trifluroacetic acid; **DNA,** deoxyribonucleic acid; **GSTs,** glutathione *s*-transferases; **ROS,** reactive oxygen species; **IMTech,** institute of microbial technology; **DAD,** diode array detector.

mutagens, participate in free radical generation or impart electrophile toxicities was earlier recommended by De Flora et al. (1992). Such compounds that neutralize the effect of mutagens/carcinogens may be appropriately termed as antimutagens/anticarcinogens and those that neutralize the effect of free radicals are termed as antioxidants.

These defensive chemicals are mainly secondary metabolites synthesized by plants. Among a variety of bioactive compounds belonging to different chemical groups, polyphenols including flavonoids are an important class of natural products having multiple polar functionality (Shahidi and Wanasunadra, 1992; Ramos, 2008). These phytochemicals are reported to play a variety of roles such as suppressor of tumor growth by inhibiting cell proliferation and inducing apoptosis, as antimutagens, inducer of detoxifying enzymes (phase II xenobiotic-metabolizing enzymes) including glutathione *S*-transferases (GSTs), free radical and reactive oxygen species (ROS) scavengers (Bhuvaneswari et al., 2005; Kwon and Magnuson, 2009; Goswami and Das, 2009; Harish et al., 2010). The interest in the natural-product-based drug discovery system has increased due to advancement in technologies like combinatorial synthesis and high throughput screening and related approaches used in formulation preparations and drug designing (Nobili et al., 2009; Boivin et al., 2009; Yasukawa et al., 2009).

Although, a number of plant species, enriched with diverse array of phytochemicals, from all over the world, have been explored for their antimutagenic and antioxidant properties yet the vast majority of plants have still not been adequately evaluated. Considering all the above mentioned facts, the present investigation is planned to investigate the antimutagenic potential of bark of chickrassy (*Chukrasia tabularis*) against direct and S9 dependent mutagens in TA98 and TA100 strains of *Salmonella typhimurium* and identification of its active constituents using HPLC.

The plant *C. tabularis* A. Juss. (belong to a family called meliaceae) is commonly known as chickrassy, lal devdari, chittgong wood. The bark and leaves of plant accumulates a variety of secondary metabolites including phenolic compounds, terpenes, limonoids and steroids (Kaur and Arora, 2009). The bark of *C. tabularis* has been used in Ayurvedic system of medicine as an astringent and anti- diarrheal drug (Kirtikar and Basu, 1981; Rastogi and Mehrotra, 1993).

MATERIALS AND METHODS

Plant material

The bark of plant was collected from the tree growing in the Guru Nanak Dev University campus, Amritsar. Botanical identification was made from Herbarium of Department of Botanical and Environmental Sciences, GNDU, Amritsar, where a voucher specimen (Accession No. 6422/2236 dated 7th April, 2006) was deposited.

Preparation of extract and its fractions

The bark material of *C. tabularis* were thoroughly washed with tap water, dried at room temperature and ground to fine powder. The powdered bark (1.110 kg) was extracted with 80% methanol (1500 ml × 3) by employing maceration method. The 80% methanol extract (MEB) so obtained was further fractionated using different solvents *viz.* hexane, ethyl acetate and n-butanol to obtain hexane fraction (HFB), ethyl acetate fraction (EAFB) and n-butanol fraction (BFB), respectively. The supernatant obtained was pooled and filtered after extracting in respective solvents, three times, using Whatman no. 1 sheet and concentrated by vacuum rotary evaporator (Strike 202, Stereo glass, Italy) followed by lyophilization to obtain the dry residue from the respective fractions (Flowchart 1). The yield of respective fractions is 0.024% (0.268 g) of HFB, 2.641% (29.32 g) of EAFB and 14.44% (160.33 g) of BFB of that of initial weight of bark powder taken. The extracts and fractions so obtained were analyzed for their antimutagenic activities in histidine point reversion assay.

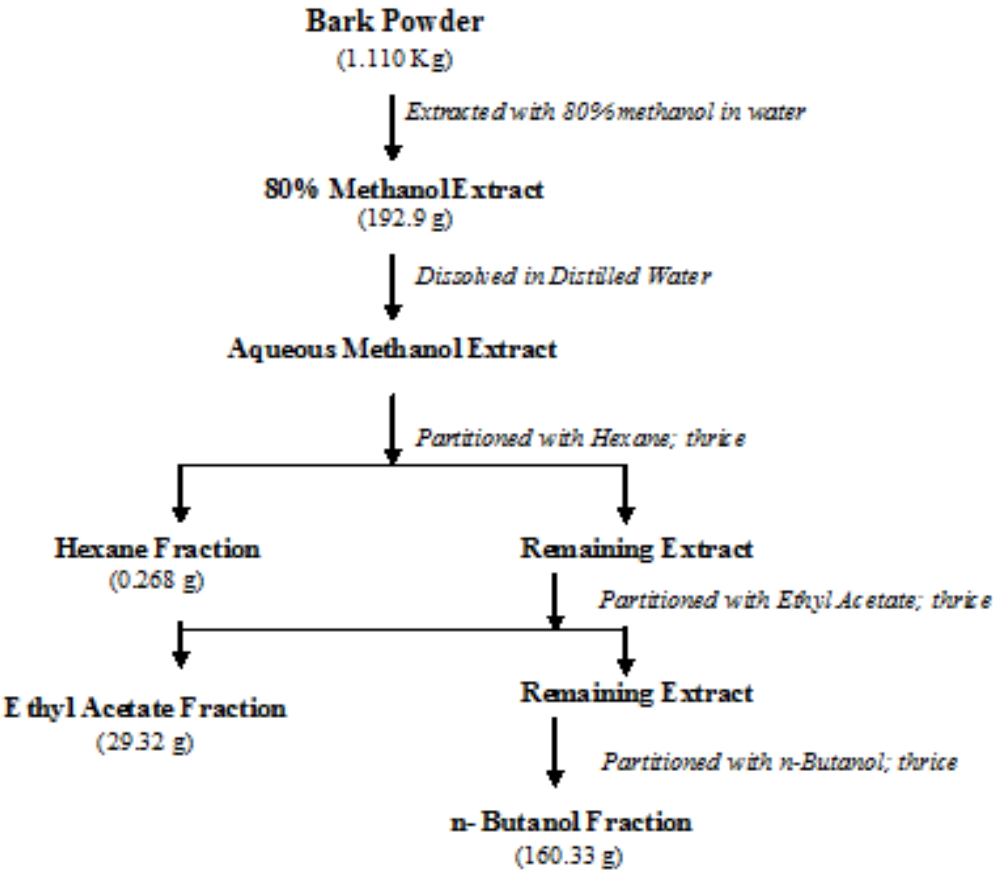
Chemicals

For HPLC analysis, gallic acid, catechin, epicatechin, rutin, 7-hydroxycoumarin (umbelliferone), quercetin, were purchased from Sigma–Aldrich and Chromadex, Life Technology, India. All HPLC grade solvents (methanol, water) were purchased from J.T. Baker (USA). Trifluoroacetic acid was purchased from E. Merck India. 4-nitro-*o*-phenylenediamine (NPD), sodium azide, 2-Aminofluorene (2-AF) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemical reagents (ethanol, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, sodium chloride, histidine, biotin, glucose, calcium chloride, potassium chloride, magnesium chloride, magnesium sulphate, ammonium chloride, Glucose-6-phosphate, reduced nicotinamide adenine dinucleotide phosphate (NADPH), agar, luria broth, etc.) and solvents (hexane, methanol, ethyl acetate, n-butanol, DMSO) were of analytical grade. Tester strains TA98 and TA100 of *Salmonella typhimurium* were procured from Institute of Microbial Technology (IMTech), Chandigarh, India.

Antimutagenic studies

The antimutagenicity of the methanol extract of *C. tabularis* bark and its different fractions was determined using *Salmonella* histidine point mutation assay proposed by Maron and Ames (1983) with slight modifications suggested by Bala and Grover (1989). All the extracts were prepared in DMSO and mutagens were dissolved in DMSO or water as per their solubility. The concentrations of extract and fractions used for investigating the antimutagenicity were 100, 250, 500, 1000, 2500 µg/0.1ml/plate. Two methodologies were followed, that is, co-incubation and pre-incubation in order to characterize antimutagenicity of compounds to either desmutagenicity or bioantimutagenicity. In co-incubation method, 100 µl each of bacterial culture, mutagen and different concentrations of extract was added to 2 ml of top agar (45°C).

In pre-incubation method, equal volume of mutagen and extract was mixed and incubated at 37°C for 30 min. 200 µl of this was added to 2 ml of top agar with 100 µl of bacterial culture. The mutagen used was 4 Nitro-o-phenylenediamine (NPD, 20 µg/0.1ml/plate) for TA98 strain and sodium azide (2.5 µg/0.1ml/plate) for TA100 strain. The top agar was then poured and evenly spread on minimal agar plates followed by incubation at 37°C for 48 h. Each concentration was tested in triplicate. Spontaneous (100 µl bacterial culture), positive control (100 µl bacterial culture + 100 µl mutagen) and negative control (100 µl



Flow chart 1. Extraction procedure for bark of *Chukrasia tabularis*.

bacterial culture+100 µl extract) were also run. There are certain carcinogens which are inactive in bacterial systems but get activated in the presence of cytochrome-based P450 metabolic activation system (Phase I), present mainly in the liver of humans and lower animals. The enzymes of mammalian oxidation system are involved in the metabolism of these procarcinogen to DNA reactive and electrophilic forms. The effect of extracts and fractions on the mutagenicity of such promutagens, 2 AF (20 µg/0.1ml/plate) in the present study, was determined using post-mitochondrial supernatant (S9 fraction) from rat liver homogenate.

Preparation of S9 fraction

The liver homogenate was prepared as per the method given by Garner et al. (1972). Male albino rats (150 to 200 g weight), was given 0.1% phenobarbitol (mixed-function oxidase inducer) in drinking water for 6 days. On the 7th day, the rats were sacrificed by cervical dislocation and livers were excised out. Livers were weighed and washed several times with chilled sterilized 0.15 M KCl solutions to ensure blood free sterile preparation. The washed livers were transferred to beakers containing chilled 0.15 M KCl solution (3 ml/g wet liver). Livers were minced and homogenized. The homogenate was centrifuged at 9000 rpm for 10 min, and supernatant was decanted off in the flask and distributed in 2 ml cryovials and stored immediately in liquid nitrogen till needed. The mutagenicity of indirect acting mutagen was checked with the addition of S-9 mix (comprising of enzymatic cofactors including NADP, $MgCl_2$, KCl, Glucose-6-Phosphate, Phosphate buffer and S9 fraction) to the top agar. S9 mix was made on the day of experiment and maintained at 0-4°C. NADP was involved in the generation of NADPH whereas phosphate buffer was necessary for stabilizing the S9 activity in top agar. Glucose-6-phosphate did not enhance mutagenesis in S9 preparation from rat liver homogenate but it resulted in slight stimulation with human liver preparation, so it was added as a cofactor in the preparation of S9 mix.

In order to estimate antimutagenicity against promutagen (2-Aminofluorene), S9 mix (0.5 ml), bacterial culture (0.1 ml), plant extract of different concentrations (100 µg/0.1ml/plate to 2500 µg/0.1ml/plate) and mutagen (0.1 ml) were mixed with soft agar and poured onto the minimal plates. After incubation for 48 h, the number of revertant *his*$^+$ bacteria colonies was scored. The negative control was run with different concentrations of extract and fractions to verify the toxicity of test sample. The concentrations were considered non-toxic if the number and size of revertant colonies in negative control were equivalent to that of spontaneous revertant colonies.

Also for non toxic effect of extract and fractions, the intensity of background lawn should be equivalent to the control having only bacterial culture. For determining the toxicity of test sample, 0.1 ml of extract or fractions of different concentrations along with 0.1 ml of freshly grown culture was added to top agar maintained at 45°C. 0.5 ml of S9 mix was also added to top agar when the negative control was run for checking the effect against indirect acting mutagen. The mixture was then plated on the minimal glucose agar plates which were then incubated at 37°C for 48 h. The antimutagenic activity of each extract and fraction was expressed as percent decrease of reverse mutations as follows:

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

Where 'x' is the number of histidine revertants induced by mutagen alone, 'y' the number of his$^+$ revertants induced by mutagen in the presence of extract and 'z' is the number of revertants induced in absence of mutagen.

Statistical analysis

The experimental data were expressed as mean (number of revertant colonies) ± SE and Inhibition (%) ± SE. One way and two way analysis of variance (ANOVA) and Tukey's HSD post hoc test were carried out to determine significant differences ($p \leq 0.05$) between the means.

High performance liquid chromatography

The methanol extract was subjected to HPLC in order to identify the presence of phenolic compounds (gallic acid, catechin, epicatechin, umbelliferone, quercetin and rutin). The preparation of sample and other conditions in order to identify active constituents are mentioned herewith:

Sample preparation

For HPLC analysis, 100 mg of bark powder was taken and extracted with methanol (25 ml × 3); thrice. The supernatant was collected and dried on rotary vacuum evaporator at 40˚C. The resultant dried extract was dissolved in solvent (methanol (90): water (10)) and analyzed for the presence of known standard compounds using the linear gradient elution method.

Preparation of standard solution

Standard stock solution of phenol was prepared by dissolving gallic acid (1mg), catechin (2 mg), epicatechin (3 mg), umbelliferone (1 mg), quercetin (5 mg) and rutin (5 mg) in 2 ml of methanol: water (90:10).

Apparatus and chromatographic conditions

HPLC analysis was performed on a Shimadzu Prominence HPLC system, equipped with LC-20AT quaternary gradient pump, SPD-M20A diode array detector (DAD), CBM-20A communication bus module, CTO-10AS VP column oven, Rheodyne injector, and Shimadzu LC solution (ver. 1.21 SP1) software. Chromatography was carried out on a Lichrocart 250-4 column from Lichrospher (250 mm×4.0 mm×5 µM particle size). At a column temperature of 27°C and a flow rate of 1 mL/min using solvent A (0.03% trifluroacetic acid (TFA) in water) and solvent B (methanol) with a linear gradient elution: 25% B (4 min), 25–40% (6 min), 40–50%B (8 min), 50–45% (12 min), 45–35% B (12 min), 35-20%B (15 min), 20-5% B (18 min), 5-25% B (20 min) at λ 280 nm.

RESULTS

Antimutagenic studies

It was found that different extract and fractions of chickrassy were non-toxic to TA98 and TA100 strains of *Salmonella typhimurium* at the all the concentrations tested (100 µg/0.1 ml/plate to 2500 µg/0.1 ml/plate). The different extracts and fractions of *C. tabularis* bark were also effective in inhibiting the mutagenicity of S9 dependent mutagens 2-AF in comparison to that of direct acting mutagens (NPD in TA98 and sodium azide in TA100 strain). These fractions exhibited lower ID_{50} values against the indirect acting mutagens and that too in pre-incubation mode of treatment. Table 1 shows that methanol extract of bark exhibited 47.89 and 47.67% inhibitory activity at the maximum dose tested (2500 µg/0.1 ml) in co-incubation and pre-incubation mode of treatment, respectively against NPD- a direct acting mutagen in TA98 strain of *S. typhimurium* whereas it reduced his$^+$ revertants induced by sodium azide in TA100 strain by 12.03 and 55.23% in co-incubation and pre-incubation mode of treatment, respectively. These results showed that MEB was ineffective against sodium azide in co-incubation mode of treatment even at the maximum dose tested. Table 1 also indicated the efficacy of MEB to prevent the 2-AF induced mutations in TA98 strain of *S. typhimurium* with an ID_{50} value of 0.358 µg/0.1ml in pre-incubation mode of treatment as compared to ID_{50} value of 121.105 µg/0.1 ml in co-incubation treatment. However, in TA100 strain, the extract exhibited an ID_{50} value of 0.007 µg/0.1ml in pre-incubation mode of treatment that was much lower than the ID_{50} values obtained in co-incubation mode of experimentation (Table 1).

However, HFB was found to be ineffective in preventing mutagenicity induced by NPD and sodium azide in TA98 and TA100 strains of *S. typhimurium* in both co-incubation and pre-incubation mode of treatments (Table 2). On the contrary, it was found that HFB effectively reduced the number of his$^+$ revertants induced by S9 dependent mutagen in TA100 strain of *S. typhimurium* as compared to TA98 strain. HFB exhibited inhibitory activity of 47.02 and 54.44% in co-incubation and pre-incubation mode of treatment, respectively at maximum concentration (2500 µg/0.1ml) tested in TA98 strain. In TA100 strain, HFB exhibited an ID_{50} value of 230.995 µg/0.1ml and 93.215 µg/0.1ml in co-incubation and pre-incubation mode of treatment, respectively (Table 2). EAFB showed almost similar antimutagenic activity against NPD, a mutagen inducing frame shift mutation in TA98 strain, in both co-incubation and pre-incubation mode of treatments with inhibitory percentage of 33.56 and 33.00%, respectively at the maximum dose tested (Table 3). However, in TA100 strain, it exhibited an inhibitory activity of 23.15 and 29.83% for sodium azide in co-incubation and pre-incubation treatments, respectively at maximum dose tested (2500 µg/0.1ml). EAFB was also found to exhibit strong inhibitory activity against S9 dependent mutagen with by 97.28% reduction in the number of his$^+$ revertants at 100 µg/0.1ml/plate dose in pre-incubation treatment.

Table 1. Effect of 80% Methanol Extract of *Chukrasia tabularis* bark on the mutagenicity of direct acting mutagens (NPD and Sodium Azide) and promutagen (2-AF) in TA98 and TA100 strains of *Salmonella typhimurium.*

Treatments	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh (%) ± SE	Mean ± SE	Inh (%) ± SE
Spontaneous		30.83 ± 1.887	-	36.5 ± 2.579	-	190.5 ± 9.96	-	229 ± 32.87	-
Positive control									
NPD	20	1199 ± 146.8^{a}	-	-	-	-	-	-	-
Sodium zide	2.5	-	-	-	-	2042.17 ± 66.45^{a}	-	-	-
2-AF	20	-	-	6756.0 ± 708.0^{a}	-	-	-	2889 ± 105.4^{a}	-
Negative control									
	100	32.00 ± 2.944	-	32.50 ± 2.277	-	185.7 ± 16.44	-	225.0 ± 32.79	-
	250	33.33 ± 2.390	-	31.00 ± 2.608	-	202.7 ± 11.67	-	230.7 ± 33.43	-
	500	35.83 ± 2.786	-	37.17 ± 3.371	-	209 ± 12.88	-	223.3 ± 30.22	-
	1000	36.00 ± 2.745	-	33.00 ± 3.367	-	194.2 ± 14.3	-	228.8 ± 32.38	-
	2500	37.67 ± 2.894	-	31.83 ± 1.922	-	197.5 ± 9.018	-	213.2 ± 32.15	-
Co-incubation									
	100	867.3 ± 81.45^{a_1}	28.42 ± 6.979	4151.0 ± 352.1^{b}	38.75 ± 5.237	2004.33 ± 36.28^{a}	2.038 ± 1.954	2798.0 ± 66.35^{a}	3.43 ± 2.491
	250	834.2 ± 90.24^{a_1a_2}	31.37 ± 7.777	2477.0 ± 93.81^{c}	63.64 ± 1.394	1979.67 ±27.1^{a}	3.40 ± 1.473	1244.0 ± 87.01^{b}	61.88 ± 3.272
	500	821.3 ± 87.57^{a_1a_2a_3}	32.47 ± 7.529	620.3 ± 108.5^{d}	91.32 ± 1.615	1853.33 ±54.02^{a}	10.28 ± 2.947	572.3 ± 59.03^{c}	86.91 ± 2.216
	1000	795.0 ± 78.65^{a_1a_2a_3a_4}	34.74 ± 6.763	258.3 ± 23.64^{de}	96.65 ± 0.351	1850.83 ± 59.9^{a}	10.35 ± 3.241	360.3 ± 32.37^{cd}	95.06 ± 1.217
	2500	642.8 ± 60.21 ^{a_1a_2a_3a_4b}	47.89 ± 5.184	30.00 ± 3.194^{def}	100.0 ± 0.048	1820.33 ± 51.97^{a}	12.03 ± 2.818	213.2 ± 34.36^{de}	97.01 ± 1.284
HSD F-ratio $_{(5,30)}$		407.1554		1411.416		311.7203		297.8991	
F-ratio $_{(5,30)}$		3.761824*		65.74095*		3.2305*		298.9729*	
Pre-incubation									
	100	857.3 ± 64.26 ^{b}	29.29 ± 5.511	1173.0 ± 118.8^{b}	83.04 ± 1.767	1781.67 ±25.89^{b}	14.03 ± 1.394	630.7 ± 42.32^{b}	84.77 ± 1.589
	250	734.5 ± 43.83 ^{bc}	39.85 ± 3.761	991.3 ±101.6^{bc}	85.72 ± 1.511	1672 ±18.48^{bc}	20.12 ± 1.003	541.5 ± 30.9^{bc}	88.31 ± 1.162
	500	728.0 ± 58.58 ^{bcd}	40.5 ± 5.036	178.5 ± 18.68^{bcd}	97.90 ± 0.278	1408.6 ± 32.58^{d}	34.50 ± 1.776	435.5 ± 40.11^{bcd}	92.04 ± 1.505
	1000	677.5 ± 51.66 ^{bcde}	44.84 ± 4.442	86.83 ± 7.635^{bcde}	99.20 ± 0.115	1237.33 ± 41.36^{de}	43.55 ± 2.239	367.8 ± 51.43^{cde}	94.77 ± 1.933
	2500	645.3 ± 22.33 ^{bcdef}	47.67 ± 1.92	33.0 ± 3.183^{bcdef}	99.98 ± 0.047	1023.33 ± 51.31^{f}	55.23 ± 2.781	319.0 ± 58.21^{cdef}	96.04 ± 2.172
HSD $_{5,30)}$		324.5803		1273.394		182.702		257.401	
F-Ratio $_{(5,30)}$		7.396948*		77.26248*		77.84053*		278.2095*	

Table 1. Contd. Two independent-way ANOVA**:** Co-Incubation and Pre-Incubation.

	TA 98 (Without S9)	TA 98 (With S9)	TA 100 (Without S9)	TA 100 (With S9)
Treatment	F-ratio$_{(1,50)}$ = 2.2521	F-ratio$_{(1,50)}$ = 151.4354*	F-ratio$_{(1,50)}$ = 155.4432*	F-ratio$_{(1,50)}$ = 45.2288*
Dose	F-ratio$_{(4,50)}$ = 2.8149*	F-ratio$_{(4,50)}$ = 148.8961*	F-ratio$_{(4,50)}$ = 24.15081*	F-ratio$_{(4,50)}$ = 9.07575*
Treatment X Dose	F-ratio$_{(4,50)}$ = 0.3433	F-ratio$_{(4,50)}$ = 314.8615*	F-ratio$_{(4,50)}$ = 241.5460*	F-ratio$_{(4,50)}$ = 153.2911*

* represents the significance at $p \leq 0.05$; Data shown are Mean ± SE of two independent experiments performed in triplicate. Means followed by same letters are not significantly different using HSD Multiple comparison test.

However, for TA98 strain, the inhibitory activity of EAFB was found to be 73.24 and 89.60% at 500 µg/0.1 ml, respectively with an ID_{50} value of 348.178 µg/0.1ml and 80.794 µg/0.1 ml, as calculated from regression equation, in co-incubation and pre-incubation treatments, respectively (Table 3).

The BFB was also found to be effective in reducing frame shift mutations induced by NPD in TA98 strain but at higher concentrations. As clear from Table 4, BFB exhibited an inhibitory activity of 28.41 and 43.91% in co-incubation and pre-incubation treatments, respectively in TA98 strain. It showed 18.84 and 27.78% inhibition in co-incubation and pre-incubation mode of treatments, respectively against sodium azide induced mutagenicity. The antimutagenic effect of BFB was more pronounced against promutagen with 90.65 and 97.22% reduction in the number of histidine revertants during co-incubation and pre-incubation mode of experiments, respectively at the dose of 250 µg/0.1ml/plate in TA100 strain of *S. typhimurium*. For TA98 strains, BFB showed an ID_{50} value of 173.883 µg/0.1 ml in co-incubtion and 8.817 µg/0.1 ml in pre-incubation mode of treatments against indirect acting mutagen. From the present study, it was found that methanol extract and ethyl acetate fraction of bark was most effective. The results of one way ANOVA and Tukey's HSD post hoc test revealed that the antimutagenic effect obtained against direct and indirect acting mutagens were statistically significant at $p \leq 0.05$. The statistically significant differences were also observed between the results obtained for two modes of treatments that is, co-incubation and pre-incubation as evaluated using (Tables 1 to 4).

HPLC analysis

Figure 1 shows the retention time for the phenolic compounds that is, gallic acid, catechin, epicatechin, umbelliferone, rutin and quercetin. On the basis of retention time and comparing the chromatogram obtained for the methanol extract of *C. tabularis* bark, it was concluded that the bark of *C. tabularis* contained Catechin (RT = 6.534 min.), Quercetin (RT= 14.171) and Rutin (RT = 16.245) (Figure 2).

DISCUSSION

Hung and co-workers (2009) classified inhibitors of mutagenesis into three categories on the basis of their mode of action. As bioantimutagenic agents, the chemopreventive agents modulate the cellular mutagenic processes by acting on DNA replication and repair processes. These agents exert their effect when DNA is damaged by the mutagen. In co-incubation method, the bioantimutagenic effect of phytochemicals is determined. The phytochemicals might be involved in the direct inactivation of mutagens or suppressed the activity of metabolic enzymes in order to convert procarcinogens to carcinogenic form. Such type of inhibition is known as desmutagenesis and pre-incubation treatment is destined to evaluate the desmutagenic effect. There is another category of antimutagens that exert its effect by acting as blocking agents. These agents act differentially from bioantimua-tegnic and desmutagenic agents as they modify the function of bacterial cells in order reduce the DNA mutations induced by mutagens (Hung et al., 2009). The extract and fractions of different plants are reported to possess effective antimutagenicity and it has been seen that desmutagenicity or bioantimutagenicity are the probable mode of action of these metabolites. In order to evaluate desmutagenicity in the current study, test sample was incubated with mutagen at 37˚C for 30 min prior to addition of bacterial culture. The bioantimutagenic effect of extract and fractions was determined by simultaneous addition of sample, mutagen and bacterial culture to the top agar that was then uniformly spread to minimal agar plates.

The effect against indirect acting mutagens was determined in the presence of S9 mixture. It was clear from the present study that the extract and fractions of *C. tabularis* bark showed more

Table 2. Effect of hexane fraction of *Chukrasia tabularis* bark on the mutagenicity of direct acting mutagens (NPD and Sodium Azide) and promutagen (2-AF) in TA98 and TA100 strains of *Salmonella typhimurium.*

Treatments	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh (%) ± SE	Mean ± SE	Inh (%) ± SE
Spontaneous		32.33 ± 2.728	-	43.33 ± 4.667	-	195.3 ± 12.14	-	196.0 ± 21.38	-
Positive control									
NPD	20	1492.0 ± 10.27^{a}	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1935.0 ± 55.56^{a}	-	-	-
2-AF	210	-	-	4845.0 ± 238.1^{a}	-	-	-	2645.0 ± 74.05^{a}	-
Negative control									
	100	32.00± 2.646	-	40.67 ± 6.566	-	182.7 ± 13.74	-	166.3 ± 32.59	-
	250	33.67 ± 4.096	-	38.00 ± 3.464	-	172.3 ± 16.33	-	184.3 ± 6.119	-
	500	34.00 ± 3.786	-	34.33 ± 3.283	-	176.0 ± 11.02	-	186.7 ± 11.29	-
	1000	33.67 ± 1.202	-	39.33 ± 5.925	-	181.3 ± 11.1	-	176.0 ± 14.57	-
	2500	36.0 ± 5.292	-	39.00 ± 7.937	-	181.3 ± 19.81	-	188.7 ± 11.41	-
Co-incubation									
	100	1474.0 ± 15.01^{a_1}	1.233 ± 1.027	4511.0 ± 113.8^{a_1}	6.947 ± 2.37	1866.0 ± 57.2^{a_1}	4.263 ± 3.504	1724.0 ± 38.28^{b}	37.14 ± 1.546
	250	1404.0 ± 3.844 ^{a_1b}	6.057 ± 0.262	4377.0 ± 231.2^{a_1a_2}	9.73 ± 4.81	1766.0 ± 20.22^{a_1a_2}	9.57 ± 1.147	1565.0 ± 72.89^{bc}	43.9 ± 2.961
	500	1369.0 ± 9.615^{bc}	8.413 ± 0.661	3882.0 ± 80.56 ^{a_1a_2b}	20.02 ± 1.675	1679.0 ± 33.59 ^{a_1a_2b}	14.54 ± 1.908	1061.0 ± 115.8^{d}	64.45 ± 4.712
	1000	1287.0 ± 15.93^{cd}	14.03 ± 1.091	3435.0 ± 179.2^{bc}	29.33 ± 3.73	1668.0 ± 64.88 $^{a_1a_2b\,c}$	15.21 ± 3.698	503.3 ± 26.18^{e}	86.74 ± 1.061
	2500	1235.0 ± 35.88^{de}	17.63 ± 2.463	2585.0 ± 117.9^{d}	47.02 ± 2.45	1633.0 ± 20.21 $^{a_2b\,c\,d}$	17.24 ± 1.155	355.3 ± 13.28^{ef}	93.22 ± 0.543
HSD F-ratio $_{(5,30)}$		86.26382		812.627		216.9167		315.9097	
F-ratio $_{(5,12)}$		31.28436*		23.4335*		7.019094*		164.6818*	
Pre-incubation									
	100	1446.0 ± 27.59^{a_1}	3.427 ± 1.635	4265.0 ± 119.6	12.70 ± 2.492	1826.0 ± 36.32 ^{a_1}	6.203 ± 2.072	1396.0 ± 45.33^{b}	50.38 ± 1.831
	250	1387.0 ± 15.07 ^{a_1b}	7.177 ± 1.034	3892.0 ± 34.08	19.83 ± 0.71	1729.0 ± 27.09 ^{a_1b}	11.67 ± 1.538	1235.0 ± 65.8^{bc}	57.3 ± 2.672
	500	1287.0 ± 17.33^{c}	14.08 ± 1.19	3280.0 ± 147.6	32.53 ± 3.069	1571.0 ± 24.23^{bc}	17.64 ± 1.279	508.3 ± 21.42^{d}	86.92 ± 0.872
	1000	1199.0 ± 25.98^{cd}	20.12 ± 1.781	2601.0 ± 163.5	46.69 ± 3.403	1560.0 ± 33.34^{bcd}	21.4 ± 1.901	388.0 ± 14.57^{de}	91.41 ± 0.590
	2500	1087.0 ± 15.07^{e}	27.79 ± 1.036	2228.0 ± 161.5	54.44 ± 3.361	1532.0 ± 32.02^{cde}	22.98 ± 1.826	304.7 ± 13.38^{def}	95.28 ± 0.544
HSD F-ratio $_{(5,30)}$		92.93011		742.9549		171.0867		218.6816	
F-ratio $_{(5,12)}$		62.70102*		41.02738*		19.39174*		375.4702*	

Table 2. Contd. Two independent-way ANOVA, Co-Incubation and Pre-Incubation.

	TA 98 (Without S9)	TA 98 (With S9)	TA 100 (Without S9)	TA 100 (With S9)
Treatment	F-ratio$_{(1,20)}$ = 32.5382*	F-ratio$_{(1,20)}$ = 30.488*	F-ratio$_{(1,20)}$ = 8.2334*	F-ratio$_{(1,20)}$ = 66.9269*
Dose	F-ratio$_{(4,20)}$ = 69.4279*	F-ratio$_{(4,20)}$ = 62.8047*	F-ratio$_{(4,20)}$ = 16.4928*	F-ratio$_{(4,20)}$ = 217.4691*
Treatment X Dose	F-ratio$_{(4,20)}$ = 3.4443*	F-ratio$_{(4,20)}$ = 1.2381	F-ratio$_{(4,20)}$ = 0.41098	F-ratio$_{(4,20)}$ = 7.0046*

*Represents the significance at $p \leq 0.05$; Data shown are Mean ± SE of experiment performed in triplicate, Means followed by same letters are not significantly different using HSD Multiple comparison test.

Table 3. Effect of ethyl acetate fraction of *Chukrasia tabularis* bark on the mutagenicity of direct acting mutagens (NPD and Sodium Azide) and promutagen (2-AF) in TA98 and TA100 strains of *Salmonella typhimurium*.

Treatments	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh (%) ± SE	Mean ± SE	Inh (%) ± SE
Spontaneous		37.0 ± 3.152	-	32.67 ± 1.745	-	187.8 ± 11.41	-	160.5 ± 6.238	-
Positive control									
NPD	20	1148.0 ± 123.5^{a}	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	2147.0 ± 63.17^{a}	-	-	-
2-AF	20	-	-	7439.0 ± 633.9^{a}	-	-	-	1797.0 ± 241.6^{a}	-
Negative control									
	100	31.83 ± 2.358	-	30.67 ± 1.961	-	199.0 ± 10.5	-	148.5 ± 8.835	-
	250	34.17 ± 2.136	-	31.33 ± 1.453	-	205.3 ± 13.67	-	138.0 ± 5.882	-
	500	35.33 ± 2.445	-	33.0 ± 1.983	-	185.8 ± 4.778	-	143.7 ± 4.759	-
	1000	33.83 ± 1.922	-	33.0 ± 1.77	-	181.8 ± 17.55	-	148.2 ± 10.55	-
	2500	37.5 ± 3.052	-	31.83± 2.522	-	198.3 ± 14.48	-	138.7 ± 8.365	-
Co-incubation									
	100	1006.0 ± 77.32^{a}	12.69 ± 6.927	6709.0 ± 380.3^{a_1}	9.86 ± 5.133	2072.0 ± 61.15^{a_1}	3.875 ± 3.138	854.5 ± 107.8^{b}	57.17 ± 6.54
	250	997.3 ± 125.7^{a}	14.29 ± 11.64	5301.0 ± 489.2 ^{a_1b}	28.87 ± 6.605	1988.0 ± 39.86 ^{a_1a_2}	8.172 ± 2.052	260.5 ± 17.00^{c}	92.62 ± 1.024
	500	899.8 ± 110.3^{a}	22.30 ± 9.914	2015.0 ± 138.4^{c}	73.24 ± 1.869	1963.0 ± 44.37^{a_1a_2a_3}	9.38 ± 2.264	149.7 ± 10.15^{cd}	99.64 ± 0.614
	1000	871.7 ± 108.3^{a}	24.80 ± 9.722	430.2 ± 54.94^{d}	94.64 ± 0.742	1935.0 ± 30.77 ^{a_1a_2a_3a_4}	10.8 ± 1.566	149.3 ± 9.404^{cde}	99.65 ± 0.744
	2500	775.3 ± 75.2^{a}	33.56 ± 6.772	53.17 ± 8.689^{de}	99.71 ± 0.117	1696.0 ± 28.85$_{2}^{a_3a_4b}$	23.15 ± 1.48	141.0 ± 7.528^{cdef}	99.86 ± 0.454
HSD F-ratio $_{(5,30)}$		452.9965		1578.007		314.7866		466.4003	
F-ratio $_{(5,30)}$		1.512154		77.8374*		5.545587*		37.66614*	

Table 3. Contd.

Pre-incubation								
	100	1037.0 ± 131.4^{a}	9.992 ± 11.78	4368.0 ± 174.2^{b}	41.46 ± 2.351	1993.0 ± 66.85 ^{a_1}	7.93 ± 3.431	194.7 ± 6.042^{b}	97.28 ± 0.423
	250	882.8 ± 90.13^{a}	23.81 ± 8.092	1545.0 ± 123.1^{c}	79.58 ± 1.662	1986.0 ± 20.9 ^{a_1a_2}	8.292 ± 1.077	181.2 ± 9.765^{bc}	97.40 ± 0.589
	500	877.0 ± 99.06^{a}	24.36 ± 8.902	803.2 ± 68.13^{cd}	89.60 ± 0.921	1794.0 ± 44.1 ^{a_1a_2b}	17.99 ± 2.248	166.7 ± 10.91^{bcd}	98.61 ± 0.660
	1000	852.2 ± 101.6^{a}	26.55 ± 9.122	75.33 ± 9.294^{de}	99.43 ± 0.125	1619.0 ± 30.04^{bc}	26.88 ± 1.529	163.2 ± 16.52^{bcde}	99.09 ± 1.002
	2500	781.5 ± 93.41^{a}	33.00 ± 8.412	44.83 ± 4.52^{def}	99.82 ± 0.061	1566.0 ± 37.5 ^{cd}	29.83 ± 1.924	126.5 ± 12.8^{bcdef}	100.0 ± 0.772
HSD F-ratio $_{(5,30)}$		462.8546		1180.307		201.3006		426.644	
F-Ratio $_{(5,30)}$		1.590734		115.4496*		24.11709*		45.08395*	

Table 3. Contd. Two independent-way ANOVA, Co-Incubation and Pre-Incubation.

	TA 98 (Without S9)	TA 98 (With S9)	TA 100 (Without S9)	TA 100 (With S9)
Treatment	F-ratio$_{(1,50)}$ = 0.1375	F-ratio$_{(1,50)}$ = 128.9452*	F-ratio$_{(1,50)}$ = 19.9541*	F-ratio$_{(1,50)}$ = 41.1139*
Dose	F-ratio$_{(4,50)}$ = 1.5481	F-ratio$_{(4,50)}$ = 237.2398*	F-ratio$_{(4,50)}$ = 17.7039*	F-ratio$_{(4,50)}$ = 41.0624*
Treatment X Dose	F-ratio$_{(4,50)}$ = 0.1427	F-ratio$_{(4,50)}$ = 25.7705*	F-ratio$_{(4,50)}$ = 1.04495	F-ratio$_{(4,50)}$ = 32.5863*

* represents the significance at $p \leq 0.05$; Data shown are Mean ± SE of two independent experiments performed in triplicate, Means followed by same letters are not significantly different using HSD Multiple comparison test.

desmutagenic effect than bioantimutagenic effect in a dose dependent manner. The methanol extract of *C. tabularis* bark was found to be most active in TA98 strain while ethyl acetate fraction was found to be effective in TA100 strain against both direct and indirect acting mutagens. This differential behaviour highlights the fact that the methanol extract was effective in preventing the frame shift mutations whereas ethyl acetate fraction was potent in preventing mutations that involve base pair substitutions. These observations are in agreement with the earlier reports of Ham and co-workers (2009) that observed the antimutagenic effect of subfractions of chaga mushroom (*Inonotus obliquus*) extract against direct acting mutagens (MNNG and 4NQO) and indirect acting mutagens (Trp-P-1 and B(α)P) in TA98 and these subfractions strongly inhibited the mutagenesis induced in TA100 strain by the direct acting mutagen by approximately 80% at 50 µg/plate concentration. However, these fractions were found to have low activity against Trp-P-1 and B(α)P in TA98 strain as compared to TA100 strain which showed that these fractions were more effective in preventing base pair substitution in TA100 strain rather than preventing frame shift mutations in TA98 strain. It was found that *Inonotus obliquus* exhibited the antimutagenic activity against direct and indirect acting mutagens in dose dependent manner (Ham et al., 2009). Earlier, Kim and co-workers (2008) reported that the pretreatment of hepatoma H significantly reduced the hepatotoxicity induced by epG2 cells with immature plum extracts Benzo[a]Pyrene. The inhibitory effect was due to the induction of phase I enzymes (CYP1A1) and enhancement of glutathione-S-transferase activities by chlorogenic acid and its derivatives that were reported to detoxify the electrophilic intermediates formed on interaction with phase I enzymes (Kim et al., 2008). The possible mechanism of actions of these metabolites in Ames test include the inhibition of Phase I enzyme that is, cytochrome P-450 which causes the bioactivation of various promutagens to mutagenic forms. The main driving force involved in the inhibition of microsomal enzyme system is the formation of hydrogen bond between ketoimide group of proteins (cytochrome P450 enzymes) and hydroxyl groups of phenolic compounds that Furthermore prevent the conversion of promutagenic forms to mutagenic one TA100 strains of *S. typhimurium*. It was found that (Lee et al., 1994; Usia et al., 2005). The

Table 4. Effect of n-butanol fraction of *Chukrasia tabularis* bark on the mutagenicity of direct acting mutagens (NPD and Sodium Azide) and promutagen (2-AF) in TA98 and TA100 strains of *Salmonella typhimurium*.

Treatments	Dose (µg/0.1 ml)	TA98				TA100			
		Without S9		With S9		Without S9		With S9	
		Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh. (%) ± SE	Mean ± SE	Inh (%) ± SE	Mean ± SE	Inh (%) ± SE
Spontaneous		37.17 ± 3.114	-	47.0 ± 5.447	-	229 ± 14.01	-	191.8 ± 9.446	-
Positive control									
NPD	20	1588.0 ± 57.37^{a}	-	-	-	-	-	-	-
Sodium Azide	2.5	-	-	-	-	1874.0 ± 41.02^{a}	-	-	-
2-AF	20	-	-	4874.0 ± 249.4^{a}	-	-	-	2658.0 ± 285.7^{a}	-
Negative control									
	100	30.67 ± 2.186	-	31.67 ± 1.961	-	200.7 ± 14.8	-	165.7 ± 9.552	-
	250	35.17 ± 2.151	-	33.17 ± 2.04	-	191.3 ± 15.32	-	168.5 ± 10.04	-
	500	34.17 ± 2.428	-	34.0 ± 2.875	-	194.5 ± 16.53	-	158.7 ± 7.274	-
	1000	38.0 ± 2.671	-	35.83 ± 2.822	-	204.0 ± 16.48	-	168.3 ± 10.34	-
	2500	31.33 ± 1.647	-	36.67 ± 5.602	-	195.5 ± 11.49	-	161.2 ± 6.916	-
Co-incubation									
	100	1530.0 ± 39.25^{a_1}	3.733 ± 2.52	3127.0 ± 127.0^{b}	36.08 ± 2.622	1842.0 ± 32.85^{a_1}	1.912 ± 1.963	1406.0 ± 114.3^{b}	50.25 ± 4.584
	250	1499.0 ± 42.51^{a_1a_2}	5.72 ± 2.737	2679.0 ± 174.1^{bc}	45.34 ± 3.596	1696.0 ± 62.32^{a_1a_2}	10.56 ± 3.704	405.0 ± 34.09^{c}	90.65 ± 1.453
	500	1439.0 ± 53.07^{a_1a_2a_3}	9.59 ± 3.416	516.5 ± 90.46^{d}	90.06 ± 1.85	1697.0 ± 23.72^{a_1a_2a_3}	10.56 ± 1.412	229.2 ± 21.09^{cd}	97.18 ± 0.844
	1000	1361.0 ± 47.1 ^{a_1a_2a_3b}	14.65 ± 3.04	86.67 ± 8.065^{de}	98.95 ± 0.166	1609.0 ± 26.64^{a_2a_3b}	15.89 ± 1.596	204.2 ± 7.002^{cde}	98.56 ± 0.282
	2500	1146.0 ± 67.0^{bc}	28.41 ± 4.304	48.83 ± 2.774^{def}	99.75 ± 0.057	1558.0 ± 81.95 ^{a_2a_3bc}	18.84 ± 4.882	174.5 ± 8.065^{cdef}	99.47 ± 0.323
HSD		223.1807		600.1939		212.3502		544.8626	
F-ratio $_{(5,30)}$		9.299508*		201.0809*		6.411083*		62.68044*	
Pre-incubation									
	100	1305.0 ± 44.37^{b}	18.19 ± 2.849	1769.0 ± 128.5^{b}	64.12 ± 2.654	1581.0 ± 39.13^{b}	17.51 ± 2.339	998.3 ± 42.85^{b}	66.59 ± 1.719
	250	1196.0 ± 25.11^{bc}	25.26 ± 1.617	354.7 ± 67.1^{c}	93.36 ± 1.386	1554.0 ± 32.08^{bc}	19.02 ± 1.904	237.7 ± 15.49^{c}	97.22 ± 0.535
	500	1112.0 ± 31.03^{bcd}	30.67 ± 1.997	160.8 ± 12.55^{cd}	97.38 ± 0.259	1514.0 ± 22.46^{bcd}	21.44 ± 1.338	230.7 ± 15.49^{cd}	97.12 ± 0.619
	1000	1047.0 ± 77.99^{cde}	34.91 ± 5.032	42.67 ± 5.084^{cde}	99.86 ± 0.105	1514.0 ± 20.65^{bcde}	21.56 ± 1.237	197.2 ± 10.13^{cde}	98.84 ± 0.407
	2500	904.5 ± 39.47^{def}	43.91 ± 2.535	35.67 ± 2.741^{cdef}	100.0 ± 0.055	1408.0 ± 39.37^{def}	27.78 ± 2.344	168.3 ± 7.531^{cdef}	99.72 ± 0.301
HSD		211.3494		507.0326		143.9386		508.6932	
F-ratio $_{(5,30)}$		23.15851*		263.4351*		22.40322*		69.67778*	

Table 4. Contd. Two independent-way ANOVA, Co-Incubation and Pre-Incubation.

	TA 98 (Without S9)	TA 98 (With S9)	TA 100 (Without S9)	TA 100 (With S9)
Treatment	F-ratio$_{(1,50)}$ = 82.7554*	F-ratio$_{(1,50)}$ = 221.0779*	F-ratio$_{(1,50)}$ = 38.3680*	F-ratio$_{(1,50)}$ = 19.9991*
Dose	F-ratio$_{(4,50)}$ = 18.8406*	F-ratio$_{(4,50)}$ = 298.1430*	F-ratio$_{(4,50)}$ = 7.8644*	F-ratio$_{(4,50)}$ = 223.1411*
Treatment X Dose	F-ratio$_{(4,50)}$ = 0.4384	F-ratio$_{(4,50)}$ = 66.2686*	F-ratio$_{(4,50)}$ = 1.05621	F-ratio$_{(4,50)}$ = 9.10792*

* represents the significance at $p \leq 0.05$; Data shown are Mean ± SE of two independent experiments performed in triplicate, Means followed by same letters are not significantly different using HSD Multiple comparison test

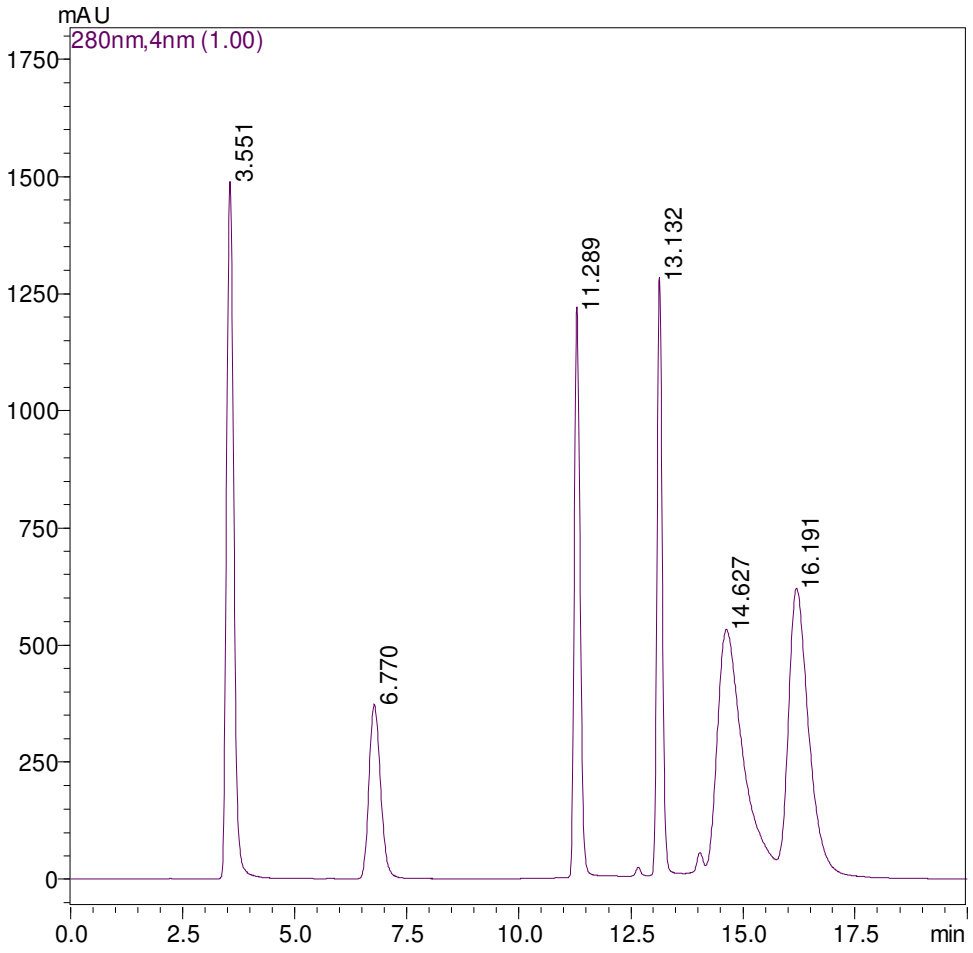


Figure 1. Chromatogram showing retention time of standard phenols that is, gallic acid (peak 1), catechin (peak 2), epicatechin (peak 3), umbelliferone (peak 4), quercetin (peak 5) and rutin (peak 6).

other possible explanation might be the involvement of different extract and fractions in scavenging of mutagenic electrophiles that are metabolically generated and thus prevent the process of mutagenesis. The pronounced activity of extract and fractions in preincubation mode of treatment against 2-AF supports these two mechanisms as either both extract and fractions prevent the conversion of 2-Aminoflorene to N-hydroxy-2-aminofluorene or inactivate the latter by binding with it that have ability to interact with DNA and thus induce mutations. However, against direct acting mutagens including sodium azide and NPD, the inhibitory activity might be due to another mechanism which involves the blocking of the mutagen transfer into the cytosol. This blocking activity might be due to phenols which act as multidentate ligands and thus are able to bind to the transporters of the outer membrane of cell at more than one point and thus form reversible complex

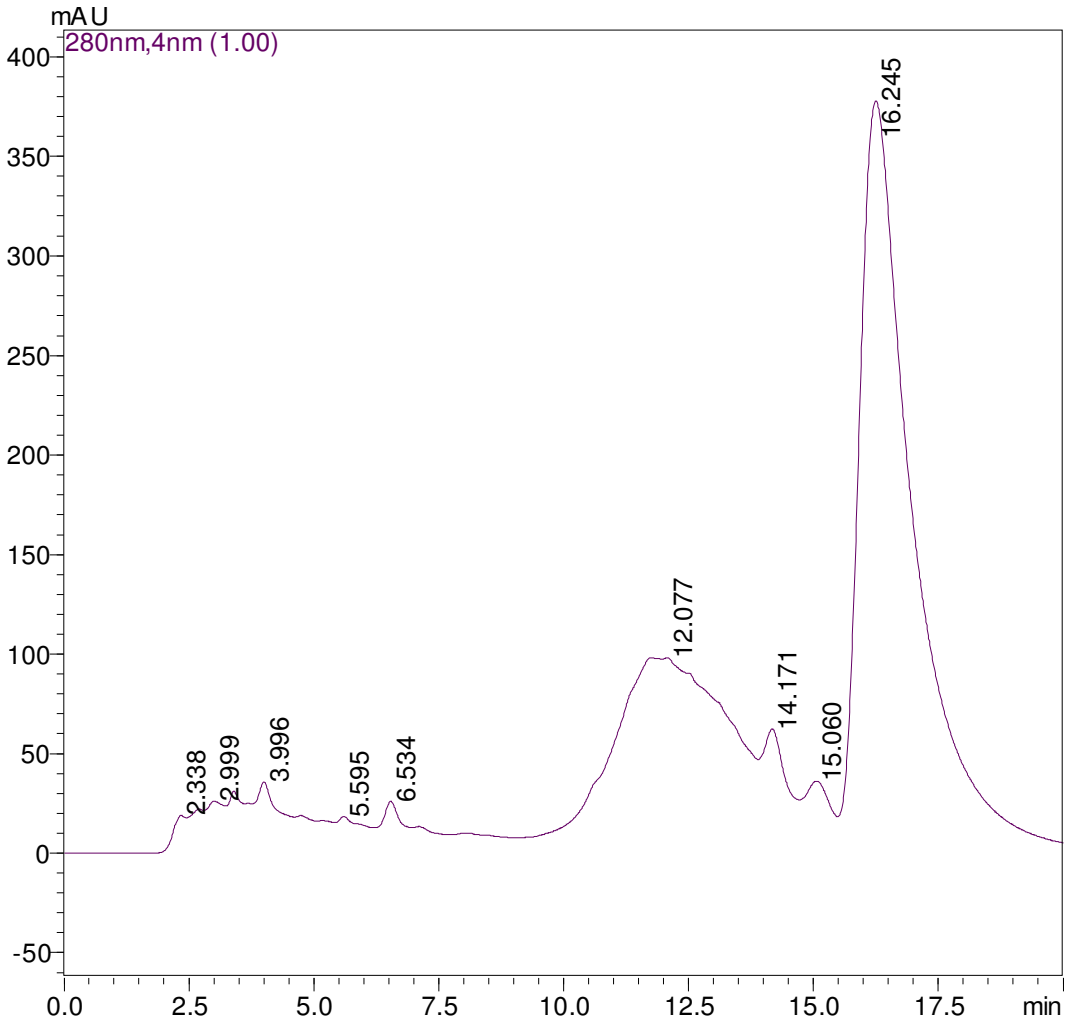


Figure 2. Chromatogram of methanol extract of *Chukrasia tabularis* bark.

hydrophobic interactions (Haslam, 1996). HPLC analysis of methanol extract of bark showed the presence of catechin, quercetin and rutin. Higher protective effect of crude extract as found in bark might be due to synergistic interaction of phenolic and steroidal compounds present in them. Besides these sterols and phenolic compounds, the presence of limonoids and phragmalin derivatives was reported by Nakatani et al. (2004); Zhang et al. (2007a, b, 2008a, 2008b); Luo et al. (2009) and these metabolites might have predominant role in imparting antimutagenic activity to these extracts and fractions of bark .

Conclusion

From the present study, a conclusion can be made that different extract and fractions of *C. tabulasris* bark exhibited significant antimutagenic activity against promutagen, 2-Aminofluorene as compared to direct acting mutagens (NPD and sodium azide) in TA98 and TA100 strains of *S. typhimurium*. These extracts and fractions has shown pronounced effect in pre-incubation mode of treatment that pointed out toward their efficacy to inhibit the activity of Phase I enzyme that is, cytochrome P-450 that causes the bioactivation of various promutagens to mutagenic forms. The HPLC analysis of crude extract that is, methanol extract led to the conclusion that the antimutagenic effect of these extract and fractions are related to the presence of catechin, quercetin and rutin.

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