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Original Article

ANTIMUTAGENIC POTENTIAL OF POLLEN GRAINS OF SOME MEDICINAL PLANT SPECIES

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ABSTRACT

Objective: The present study was planned to explore the antimutagenic response of ethanolic extracts of pollen grains of four plant species *viz., Bauhinia variegata, Cassia biflora, Cassia glauca* and *Cassia siamea* belonging to Fabaceae family.

Methods: Ames assay was used to evaluate the antimuatagenic activity of the ethanolic extracts of pollen grains of four plant species. Both TA 98 and TA 100 strains of *Salmonella typhimurium* were used in presence and absence of S9 mix during the present study.

Results: Among four species studied, pollen extracts of *Bauhinia variegata* and *Cassia biflora* had shown maximum percentage inhibition of revertant colonies during presence and absence of S9 mix, respectively.

Conclusion: The present study reveals that pollen extract of four plant species *viz., Bauhinia variegata, Cassia glauca, Cassia biflora and Cassia siamea* exhibited antimutagenic potential against two direct acting mutagens *viz.,* (4 nitro-o-phenylenediamine; NPD for TA 98 and sodium azide for TA 100) and one indirect acting mutagen (2 amino-flourine; 2AF) which indicates that pollen grains of these species can act as potential source of anticancer drugs.

Keywords: Ames assay, *Bauhinia variegata,* Pharmaceutical, Cancer

INTRODUCTION

Various damages to the genetic materials such as gene mutations, changes in number and structure of chromosomes can ultimately lead to cancer [1]. Damages to the genetic material are caused by different mutagens or carcinogens. Most of these mutagens are present in the environment itself which include chemical carcinogens and radiations. However, some other carcinogens enter the environment either by natural causes or anthropogenic activities [2-3]. These mutagens cause oxidative stress which leads to formation of reactive oxygen species such as superoxide anion radicals, hydroxyl radicals, hydrogen peroxide in human body [4]. Due to oxidative stress, human body releases more reactive oxygen species that result in homeostatic imbalance in the body and can cause cell damage [4-5].

Nowadays, exposure to these mutagens by human body is unavoidable. However, intake of antioxidants from medicinal plants can reduce the risk of these diseases. Some scientists are looking for natural foodstuffs which have antioxidant properties. Considering this, tremendous work has now been carried out all over the world to explore the antioxidant and antimutagenic potential of medicinal plants [6-8].

It is widely accepted that antioxidants present in medicinal plants play an important role in reduction of oxidative stress. The medicinal plants possess various secondary metabolites such as flavonoids, phenolics and terpenoids compounds which may reduce or inhibit the mutagenic potential of mutagens. Therefore, it becomes important to explore more plants/plant parts possessing antimutagenic properties [6-9]. The antimutagenic potential of various plant species have been explored using number of bioassays [10-11]. Among different bioassays, Ames assay is widely used and accepted bioassay to explore the antimutagenic potential of various plant species [6-7, 12].

The present study focuses on the antimutagenic potential of pollen grains of four species *viz., Bauhinia variegata, Cassia biflora, Cassia glauca and Cassia siamea* belonging to fabaceae family. Traditionally, *Bauhinia variegata* has been used to cure number of diseases such as piles, diarrhoea, dysentery, oedema, constipation, antidote for snake bite, haemorrhoids [13]. Nowadays, different parts of *Bauhinia variegata* also been explored for antibacterial [14], anti-inflammatory, antimutagenicity [15]. *C. biflora* has been reported to have different phytochemicals such as flavonoids, phenols, proteins, sapnonins, terpenoids etc. [16]. Gupta *et al.,* [17] reported that leaves of *C. glauca* showed presence of different phytochemicals *viz.,* glycosides, carbohydrates, phenolic compounds, tannins, alkaloids etc. Majji *et al.,* [18] reported the antibacterial activity of *C. siamea*. The pollen grains of various plant species have been reported to possess various bioactive compounds such as flavonoids, terpenoid, and polyphenols [19-22]. These bioactive compounds have been well documented for their different bioactivities such as antifungal, antibacterial, antioxidant, antimutagenicity, anti-inflammatory [19]. Considering this, the present study was planned to explore the antimutagenic potential of pollen grains of four plant species *viz., Bauhinia variegata, Cassia biflora, Cassia glauca and Cassia siamea.*

MATERIALS AND METHODS

Chemicals and reagents used

Different chemicals used in the study *i.e.* disodium hydrogen orthophosphate ($Na_2HPO_4.2H_2O$), potassium dihydrogen orthophosphate ($KH_2PO_4.2H_2O$), ammonium chloride (NH_4Cl), sodium chloride (NaCl), magnesium sulphate ($MgSO_4$), calcium chlorides ($CaCl_2$), glucose, histidine, biotin, agar, nicotine adenine dinucleotide phosphate, glucose-6-phosphate, $MgCl_2$ and KCl procured from Himedia Company. Two direct acting mutagens viz., (4 nitro-o-phenylenediamine; NPD for TA 98 and sodium azide for TA 100) and one indirect acting mutagen (2 amino-flourine; 2AF for both strains) were used in the experiment.

Collection of pollen grains

Four medicinal plants *viz., Bauhinia variegata, Cassia glauca, Cassia biflora and Cassia siamea* belonging to Fabaceae family were selected for the present study in order to explore the antimutagenic potential of their pollen grains. Botanical identification of different plant species were made by studying the morphological features of plants and by comparing with the herbarium sheet of the plants which were earlier submitted to the herbarium of Department of Botanical and Environmental Sciences, GNDU, Amritsar [23].

Fresh flowers (just prior to anthesis) of plant species were collected from the Guru Nanak Dev University Campus, Amritsar, Punjab

(India). For collection of pollen grains, anthers were teased with the help of sharp forceps and were tapped in pre weighted Petri plates. The weight of Petri plates with pollen was noted again. About 100-150 flowers of each plant were collected in order to obtain 1 g of pollen grains.

Preparation of pollen extracts

The ethanolic pollen extracts of all plant species were prepared by following the protocol given by Carpes *et al.* [19] with certain modifications. 70 % ethanol (7.5 ml) was added to the collected pollen grains and then extracted by 1 min shaking at interval of 10 min at 70 °C temperature for 1 h. After 1 h, the supernatant was extracted from the mixture and solid residue was re-dissolved in same volume of ethanol. The extraction was repeated till the extracts became colourless. The extracts were stored at 4 ºC till further analysis.

Estimation of antimutagenic potential of pollen extracts

Antimutagenicity of pollen extracts was estimated using Ames assay. The Ames test was performed by following the method of Moran and Ames [12] using two tester strains of *Salmonella typhimurium i.e.* TA98 and TA100. The test was carried out in the presence of S9 mix rat liver homogenate (with metabolic activation) and in absence of S9 mix rat liver homogenate (without metabolic activation).

To know the antimutagenic potential of pollen extracts against direct acting mutagens (4 nitro-o-phenylenediamine (NPD) for TA 98 and sodium azide (SA) for TA 100), 2 ml of top agar, 0.1 ml of culture (TA 98 or TA 100), 0.1 ml pollen extract, 0.1 ml mutagen (20 µg/0.1 ml/plate of 4 nitro-o-phenylenediamine for TA 98 and 2.5 µg/0.1 ml/plate sodium azide for TA 100) were added to the test mixture. To know the antimutagenic potential of pollen extracts against indirect acting mutagen (2 amino-flourine; 2AF) by metabolic activation of pollen extracts, 2 ml of top agar, 0.1 ml of culture (TA 98 or TA 100), 0.1 ml pollen extract, 0.5 ml of S9 rat liver homogenate and 2 amino-flourine (2AF; 20 µg/0.1 ml/plate) were added in test mixture. The mixture was spread on minimal agar plates. After solidification, the Petri plates were kept in the BOD incubator at 37 °C for 48 h. The number of revertant colonies was counted after 48 h.

For checking antimutagenecity, two modes of treatments *viz*., pre-incubation (PI) and co-incubation (CI) were followed. During pre-incubation, mutagen and pollen extract were pre-incubated at 37 °C for 30 min prior to their use while for co-incubation, mutagen and extract were mixed at the time of experiment.

Preparation of S9

After taking permission from ethical committee (vide no. 226/ CPCSEA2013/17 dated 24/08/2013), 5 rats (body weight: 120-150 gm approximately) were procured from Sanjay Biologicals, Amritsar. Rats were kept in animal house of Guru Nanak Dev University for 10 d for acclimatization. After acclimatization, rats were treated with 0.1% phenobarbitone for 7 d and then livers were excised from the rats.

Freshly excised livers from the rats were immediately placed in pre-weighed beakers. Livers were washed several times with the help of fresh chilled KCl and weights of livers were noted. The washed livers were transferred to sterile beakers containing chilled sterile 0.15 M KCl (3 ml/g wet liver). Livers were cut into small pieces with scissors and homogenized. The homogenate was then centrifuged at 9,000 x g (8,700 rpm) for 10 min. The supernatant (S9 fraction) was separated from pallets and distributed in 2 ml cryovials. The cryovials were immediately transferred to-80ºC till further use. For preparation of S9 mix, 16.75 ml of sterile distilled water was added in autoclaved culture tube. 25 ml of 0.2 M phosphate buffer (pH 7.4), 2 ml of 0.1 M nicotine adenine dinucleotide phosphate (NADP), 0. 25 ml of 1 M glucose-6-phosphate (G-6-P), 1 ml of $MgCl_2$-KCl salt solution and 5 ml of S9 rat liver homogenate were added to it. All the solutions were always added in the order indicated above and S9 mix was maintained at 4 °C during the whole experiments.

Statistical analysis

The results were analyzed statistically using one way and two way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Antimutagenic response of pollen extracts with and without metabolic activation is shown in tables 1-4. Pollen extracts of all plant species exhibited dose dependent response.

Table 1: Antimutagenic potential of pollen extracts of *Bauhinia variegata*

Treatment	Dose	TA 98				TA 100			
		Without S9		With S9		Without S9		With S9	
		No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)
Spontaneous	-	24.0±2.082	-	21.67±1.453	-	93.67±6.566	-	112.0±7.767	-
Positive control (µg/0.1 ml)									
NPD	20	1147±22.19	-	-	-	-	-	-	-
Sodium azide	2.5	-	-	-	-	1693±27.06	-	-	-
2AF	20	-	-	1288±12.22	-	-	-	1898±5.239	-
Negative Control									
	25%	27.67±0.333	-	25.67±1.453	-	86.67±2.028	-	105.7±5.364	-
	50%	23.00±2.517	-	24.00±1.155	-	90.33±5.487	-	118.0±4.163	-
	75%	25.33±0.882	-	22.67±1.764	-	85.67±4.177	-	110.3±5.812	-
	100%	29.00±2.309	-	26.67±1.453	-	89.00±5.132	-	114.0±2.887	-
Co-incubation									
	25%	710.7±22.19	37.07±2.044	754.7±18.67	42.25±1.429	1128±16.380	36.60±0.999	1138±36.68	42.27±2.225
	50%	630.7±7.424	44.25±0.639	669.3±19.68	48.94±1.531	884.0±40.460	50.50±2.697	992.0±26.63	50.88±1.616
	75%	575.3±16.9	49.43±1.535	496.0±34.70	62.60±2.822	829.3±11.620	53.59±0.720	764.7±35.88	63.38±2.096
	100%	437.0±26.26	62.32±2.336	257.3±28.20	81.71±2.180	777.3±57.010	57.07±3.459	532.0±49.65	76.55±2.694
F-ratio		34.7774*		70.4876*		21.167*		48.5235*	
HSD		88.3962		118.446		164.7492		172.603	
Pre-incubation									
	25%	766.7±14.11	31.91±1.308	888.3±28.42	32.19±1.904	1165±16.38	32.85±1.061	1274±32.15	34.77±1.738
	50%	669.3±5.812	40.71±0.617	698.7±27.55	46.70±2.179	1049±55.83	40.17±3.552	985.3±39.82	50.75±2.607
	75%	482.7±19.37	57.95±1.775	505.3±13.68	61.85±1.019	957.3±81.37	48.98±3.588	811.3±42.15	60.75±2.164
	100%	329.3±16.38	71.19±0.903	300.7±41.25	78.27±3.246	928.0±34.87	47.71±2.305	534.7±55.10	76.41±3.120
F-ratio		173.1792*		73.8626*		4.072402		51.75473*	
HSD		67.0548		133.1436		239.9333		195.2635	

Two way ANOVA:

Co-incubation and Pre-incubation

	TA 98 (without S9)	TA 98 (With S9)	TA 100 (without S9)	TA 98 (With S9)
Treatment	F-ratio $_{(1,16)}$ = 0.4629	F-ratio $_{(1,16)}$ = 7.5139*	F-ratio $_{(1,16)}$ = 12.5930*	F-ratio $_{(1,16)}$ = 2.4111
Dose	F-ratio $_{(3,16)}$ = 123.0168*	F-ratio $_{(3,16)}$ = 142.7909*	F-ratio $_{(3,16)}$ = 17.9221*	F-ratio $_{(3,16)}$ = 99.3912*
Treatment × Dose	F-ratio $_{(3,16)}$ = 13.6037*	F-ratio $_{(3,16)}$ = 1.9523	F-ratio $_{(3,16)}$ = 1.1772	F-ratio $_{(3,16)}$ = 1.2835

* represents the significance at p ≤ 0.05, n=3.

Table 2: Antimutagenic potential of pollen extracts of *Cassia biflora*

Treatment	Dose	TA 98				TA 100			
		Without S9		With S9		Without S9		With S9	
		No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)
Spontaneous	-	22.0±1.528	-	22.33±0.882	-	87.67±3.383	-	111.0±6.936	-
Positive control (µg/0.1 ml)									
NPD	20	1180±18.33	-	-	-	-	-	-	-
Sodium azide	2.5	-	-	-	-	1696±13.18	-	-	-
2AF	20	-	-	1295±6.96	-	-	-	1914±8.452	-
Negative Control									
	25%	23.00±2.082	-	24.67±2.028	-	93.0±2.517	-	100.3±7.535	-
	50%	23.67±0.667	-	21.33±1.453	-	94.0±4.509	-	102.0±6.083	-
	75%	22.67±1.202	-	24.33±1.202	-	83.67±2.728	-	104.0±6.557	-
	100%	26.33±2.186	-	23.0±1.155	-	82.33±8.452	-	103.3±4.485	-
Co-incubation									
	25%	766.7±12.72	35.72±1.06	1111.0±35.14	14.38±2.743	865.3±20.18	51.73±1.272	1662.0±6.119	13.83±0.357
	50%	78.0±27.01	43.41±2.322	932.0±30.02	28.44±2.420	33.3±32.36	60.02±2.054	455.0±19.54	25.28±1.161
	75%	572.0±24.98	52.53±2.106	850.3±23.38	34.84±1.919	558.7±21.83	70.48±1.348	1396.0±6.110	28.58±0.404
	100%	508.0±17.44	58.24±1.416	657.3±34.05	50.09±2.647	310.7±7.424	86.18±0.225	1233.0±4.807	37.60±0.322
F-ratio		28.7498*		36.935*		115.7872*		262.8037*	
HSD		96.6046		140.3996		100.9331		49.6064	
Pre-incubation									
	25%	722.7±23.13	39.52±1.931	1132.0±14.33	12.79±1.108	920.0±34.64	48.31±2.097	1462.0±28.21	24.94±1.420
	50%	661.3±19.64	44.85±1.676	1074.0±7.211	17.29±0.569	738.7±35.88	59.67±2.122	1153.0±57.62	38.29±3.281
	75%	518.7±24.69	57.14±2.148	862.7±26.34	33.98±2.105	582.7±39.75	68.98±2.357	1049.0±27.55	46.64±1.186
	100%	345.3±19.91	72.35±1.739	674.0±26.03	48.78±2.007	454.7±19.64	77.21±1.29	1010.0±36.80	49.89±1.913
F-ratio		58.4661*		107.5395*		36.27146*		26.9008*	
HSD		99.4309		91.4049		151.1509		178.1464	

Two way ANOVA:

Co-incubation and Pre-incubation

	TA 98 (without S9)	TA 98 (With S9)	TA 100 (without S9)	TA 98 (With S9)
Treatment	F-ratio $_{(1,16)}$ = 20.4324*	F-ratio $_{(1,16)}$ = 6.6914*	F-ratio $_{(1,16)}$ = 8.0731*	F-ratio $_{(1,16)}$ = 172.6150*
Dose	F-ratio $_{(3,16)}$ = 83.6655*	F-ratio $_{(3,16)}$ = 113.0162*	F-ratio $_{(3,16)}$ = 119.2404*	F-ratio $_{(3,16)}$ = 84.9863*
Treatment × Dose	F-ratio $_{(3,16)}$ = 4.4071*	F-ratio $_{(3,16)}$ = 2.8892	F-ratio $_{(3,16)}$ = 2.3466	F-ratio $_{(3,16)}$ = 2.7663

* represents the significance at p ≤ 0.05, n=3

Table 3: Antimutagenic potential of pollen extracts of *Cassia glauca*

Treatment	Dose	TA 98				TA 100			
		Without S9		With S9		Without S9		With S9	
		No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)
Spontaneous	-	23.33±0.882	-	23.67±1.202	-	87.67±3.383	-	112.7±5.457	-
Positive control (µg/0.1 ml)									
NPD	20	1090.0±98.03	-	-	-	-	-	-	-
Sodium azide	2.5	-	-	-	-	1696.0±13.86	-	-	-
2AF	20	-	-	1200.0±8.327	-	-	-	1891±41.38	-
Negative Control									
	25%	26.33±4.631	-	23.33±0.882	-	92.33±5.783	-	100.3±7.688	-
	50%	19.67±0.882	-	25.33±2.603	-	94.67±6.009	-	100.3±5.239	-
	75%	27.33±1.202	-	25.67±1.453	-	83.67±4.256	-	111.3±4.910	-
	100%	30.67±4.055	-	27.00±1.155	-	91.00±5.774	-	95.33±1.667	-
Co-incubation									
	25%	786.7±10.41	28.51±0.852	617.7±10.71	46.86±0.588	949.3±42.85	46.58±2.823	1252±25.56	34.59±0.562

	50%	730.0±3.606	33.63±0.364	536.3±10.2	56.50±0.980	781.3±36.54	56.88±2.237	1010±35.10	49.21±1.834
	75%	686.0±6.557	38.13±0.642	475.7±4.63	61.68±0.471	702.7±23.13	61.61±1.459	835.0±55.77	59.35±3.292
	100%	589.0±8.743	47.28±0.655	371.0±5.13	70.43±0422	525.3±17.64	72.85±0.962	550.0±35.00	74.68±1.878
F-ratio		115.3407*		161.9928*		30.8641*		56.1271*	
HSD		35.1541		36.9873		143.5692		178.6513	
Pre-incubation									
	25%	761.3±4.807	30.85±0.399	740.7±7.688	39.04±0.677	1081±19.37	38.34±1.339	1322±12.39	31.79±0.606
	50%	686.7±8.110	37.68±0.735	641.7±16.70	47.53±1.347	792.0±16.65	56.45±1.063	1104±22.06	43.97±1.239
	75%	469.3±16.38	58.60±1.651	517.3±2.728	58.08±0.314	673.0±40.55	63.44±2.654	1032±15.30	48.25±0.848
	100%	370.3±15.72	67.90±1.426	433.0±11.36	65.39±0.946	472.0±17.44	76.26±1.010	919.0±11.85	54.13±0.617
F-ratio		220.7451*		154.9193*		99.77125*		113.4734*	
HSD		55.6860		49.3298		115.5125		72.1489	

Two way ANOVA:

Co-incubation and Pre-incubation

	TA 98 (without S9)	TA 98 (With S9)	TA 100 (without S9)	TA 98 (With S9)
Treatment	F-ratio $_{(1,16)}$ = 300.4921*	F-ratio $_{(1,16)}$ = 148.7503*	F-ratio $_{(1,16)}$ = 0.5439	F-ratio $_{(1,16)}$ = 73.7140*
Dose	F-ratio $_{(3,16)}$ = 328.1444*	F-ratio $_{(3,16)}$ = 307.2856*	F-ratio $_{(3,16)}$ = 111.7861*	F-ratio $_{(3,16)}$ = 118.1121*
Treatment × Dose	F-ratio $_{(3,16)}$ = 53.2730*	F-ratio $_{(3,16)}$ = 7.6441*	F-ratio $_{(3,16)}$ = 4.0980*	F-ratio $_{(3,16)}$ = 10.2250*

* represents the significance at $p \leq 0.05$

Table 4: Antimutagenic potential of pollen extracts of *Cassia siamea*

Treatment	Dose	TA 98				TA 100			
		Without S9		With S9		Without S9		With S9	
		No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)	No. of colonies (mean±S.E.)	% inhibition (mean±S.E.)
Spontaneous	-	23.33±0.882	-	23.67±1.202	-	92.00±2.517	-	119.0±3.512	-
Positive control (µg/0.1 ml)									
NPD	20	1096.0±64.69	-	-	-	-	-	-	-
Sodium azide	2.5	-	-	-	-	1693.0±23.7	-	-	-
2AF	20	-	-	1325.0±15.07	-	-	-	1803±10.73	-
Negative Control									
	25%	20.67±1.453	-	22.33±1.764	-	94.67±5.487	-	110.3±2.963	-
	50%	26.00±2.00	-	23.33±1.453	-	91.67±3.844	-	109.3±6.741	-
	75%	24.67±0.882	-	23.00±2.082	-	89.33±4.667	-	101.7±10.27	-
	100%	24.67±2.186	-	22.33±1.764	-	82.00±3.215	-	105.7±8.172	-
Co-incubation									
	25%	722.7±21.46	36.67±1.979	869.3±12.72	34.98±0.947	952.0±18.90	46.36±1.151	1233±13.97	33.70±0878
	50%	665.0±9.018	42.07±0.855	833.3±4.807	37.77±0.357	868.0±25.72	51.52±1.604	1097±22.78	41.71±1.501
	75%	564.0±11.79	51.16±1.108	809.3±8.819	39.60±0.615	669.3±7.424	62.82±1.614	907.3±8.667	52.68±0.765
	100%	514.7±6.36	55.63±0.685	617.7±5.548	54.30±0.357	553.3±13.13	70.74±0.882	758.7±7.424	61.55±0329
F-ratio		49.4528*		172.7259*		106.4099*		206.4384*	
HSD		60.8278		38.7999		79.9587		65.8193	
Pre-incubation									
	25%	536.0±11.55	51.70±1.018	757.3±3.528	43.55±0.269	984.0±29.48	44.36±1.899	1105±10.69	41.27±0.571
	50%	464.0±12.86	60.29±1.252	703.0±5.196	47.79±0.433	781.3±22.19	57.56±1.411	1052±9.238	44.18±0.604
	75%	409.3±12.72	65.17±1.168	679.0±6.245	49.61±0.411	664.0±28.84	64.17±1.788	942.7±31.52	50.62±2.163
	100%	305.3±16.38	74.59±1.530	473.0±36.86	65.05±3.147	581.3±29.69	69.33±1.945	801.7±5.044	59.02±0.576
F-ratio		51.8666*		43.1163*		39.6909*		58.9809*	
HSD		61.1503		85.8544		125.6117		79.0770	

Two way ANOVA:

Co-incubation and Pre-incubation

	TA 98 (without S9)	TA 98 (With S9)	TA 100 (without S9)	TA 98 (With S9)
Treatment	F-ratio $_{(1,16)}$ = 389.6272*	F-ratio $_{(1,16)}$ = 154.6817*	F-ratio $_{(1,16)}$ = 0.2369	F-ratio $_{(1,16)}$ = 4.4047
Dose	F-ratio $_{(3,16)}$ = 99.7351*	F-ratio $_{(3,16)}$ = 129.7817*	F-ratio $_{(3,16)}$ = 115.0549*	F-ratio $_{(3,16)}$ = 226.1555*
Treatment × Dose	F-ratio $_{(3,16)}$ = 1.5970	F-ratio $_{(3,16)}$ = 0.41424	F-ratio $_{(3,16)}$ = 2.8048	F-ratio $_{(3,16)}$ = 12.5039*

* represents the significance at $p \leq 0.05$, n=3addition of S9 mix, the inhibitory effect of pollen extracts of *Bauhinia variegata* and *Cassia glauca* were increased.

Among all sample studied, maximum percentage inhibition of revertant colonies against 2amino-fluorine were shown by pollen extracts of *Bauhinia variegata.* During co-incubation treatment of TA 100, maximum percentage inhibition (86.18 %) of revertant colonies

was showed by pollen extract of *Cassia biflora* while minimum percentage inhibition (52.93 %) of revertant colonies was showed by pollen extract of *Bauhinia variegata* against sodium azide. The pollen extract of *Bauhinia variegata* showed maximum percentage inhibition (81.70 %) of revertants colonies while pollen extracts of *Cassia siamea* showed minimum percentage inhibition (50.09 %) of revertants colonies against NPD during co-incubation of TA 98. During pre-incubation, maximum and minimum percentage inhibitory effect against NPD was shown by *Bauhinia variegata* (78.27 %) and *Cassia biflora* (48.78 %), respectively. The pollen extract of Cassia biflora plant showed less inhibitory effects against mutagen NPD and SA in both TA 98 and TA 100 cultures. It was observed that with the

Ames test is widely used test because it is considered as most quick and convenient method to test antimutagenicity of any test compounds [7]. It is well documented that various types of bioactive compounds are present in the different parts of plants and showed their bioactivities [4,7,11,24]. Pedeschi and Cisneros-Zevallos [25] reported the antiutagenic response of phenolic fraction extracted from *Zea mays* L. Mimica-Dukic *et al.* [26] reported the antimutagenecity of essential oil from leaves of *Myrus communis* L. and screened for its antimutagenic response following Ames assay. Author reported that the antimutagenic response of plant is due to presence of 1,8-cineole and methyl eugenol compounds because these compounds are responsible for the scavenging activity of the oil. Author further stated that phenolic compounds present in the methanolic and ethanolic extracts of leaves of this plant also responsible for antimutagenic potential. Sundaram *et al.* [27] reported the antimutagenicity of ethanolic extracts of *Derris brevipes* against different mutagens *viz*., 4-nitroquinolene-1-oxide, sodium azide and 2-aminoflourene. The plant was previously used for enhancing the brain memory and concentration. Zahin *et al.* [4] analyzed leaves of *Murraya koengii* L. for their antimutagenic response. In spite of presence of bioactive compounds in other parts of the plants, the pollen grains of various plants also possess these compounds which further contribute to different bioactivities including anti-mutagenic potential of pollen grains [4, 19].

The literature survey indicated that although many reports are available on the use of various plant parts *viz*., leaves, bark, flowers of these plants to explore their bioactivities but no report is available on the use of their pollen grains. Therefore, the present study is a nobel work to explore the antimutagenic response of the pollen grains of four plant species *viz*., *Bauhinia variegata, Cassia biflora, Cassia glauca, and Cassia siamea*.

CONCLUSION

The present study reveals that pollen extract of four plant species *viz*., *Bauhinia variegata, Cassia glauca, Cassia biflora and Cassia siamea* exhibited antimutagenic potential against two direct acting mutagens *viz.,* (4 nitro-o-phenylenediamine; NPD for TA 98 and sodium azide for TA 100) and one indirect acting mutagen (2 amino-flourine; 2AF) which indicates that pollen grains of these species can act as potential source of anticancer drugs.

CONFLICT OF INTERESTS

Declared none

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