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ANTIMUTAGENIC POTENTIAL OF AQUEOUS EXTRACT OF PEELS OF TUBERS OF *Chlorophytum borivilianum* SANTAPAU AND FERNANDES

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ABSTRACT

The present study was conducted to evaluate the antimutagenic properties of aqueous extract of peels of tubers of *Chlorophytum borivilianum* which are normally considered as wastes. The antimutagenic activity of an aqueous peel extract was evaluated by employing Ames assay commonly called as plate incorporation assay. Ames assay was conducted in two modes i.e. co-incubation and pre-incubation with and without metabolic activation system (S9) by taking strains of *Salmonella typhimurium* (TA98 and TA100). The aqueous peels extract showed statistically significant decrease in mutation frequency in both the strains against all the mutagens used in the experiments.

KEY WORDS: ***Chlorophytum borivilianum*, Ames assay, mutations, antimutagens, S9.**

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INTRODUCTION

In the last few decades, scientists are searching plants or plant based molecules that can cure cancer or other diseases caused by mutagens. Antimutagenic compounds of plant origin can protect the nucleic acids (DNA or RNA) from direct acting mutagens or by inactivating the mutagenic precursors by inhibiting their transformation into carcinogens. There are several anticancer drugs that are used to cure cancer and are extracted from plants like Vinblastine and Vincristine from *Catharanthus roseus*, Podophyllotoxin from *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich, Paclitaxel *Taxus brevifolia* Nutt., Camptothecin from *Camptotheca acuminate* etc[1]. There are many scientific reports that also indicate the presence of therapeutic compounds in the plant parts that are not generally eaten or consumed by man especially peels of fruits and vegetables. Peels of Pomegranate (*Punica granatum*), Mango (*Mangifera indica*), Apple (*Malus domestica*), Orange (*Citrus sinensis*), Banana (*Musa paradisiaca*), Potato (*Solanum tuberosum*), Tori (*Luffa Cylindrica*) etc. has antimicrobial and antioxidant activities[2]. Peels of *Punica granatum* were also found to be rich in antimutagenic compounds[3,4]. Peels of fruits are generated as by product in large amount in food industries and can be utilized for the extraction of bioactive compound that have proven health benefits[5]. *Chlorophytum borivilianum* is an important aphrodisiac plant and belongs to family Liliaceae and commonly known as safed musli. It is used in Ayurvedic system of medicine to cure various ailments. Tubers of this plant are peeled off before use. In our previous study, we reported that peels of this plant have antioxidant potential and can be used for the extraction of antioxidant compounds[6]. Therefore, the present study was planned to investigate its antimutagenic activities.

MATERIALS AND METHODS

Materials

Two strains of *Salmonella typhimurium* (TA98 and TA100) were procured from the Institute of Microbial Technology (CSIR), Chandigarh, India. All the chemicals used in the experiments were of analytical grade.

Plant extract

Peels were removed from the freshly harvested tubers of *Chlorophytum borivilianum* and dried peels were extracted with water (thrice) using maceration method to obtain aqueous peel extract.

Antimutagenic testing of extract

Aqueous peel extract was checked for antimutagenic potential using Ames assay suggested by Maron and Ames[7]. Two strains of *Salmonella typhimurium* (TA98 and TA100) were used in the present investigation. Mutagen NPD (4-nitro-o-phenylene diamine) (for TA98) and sodium azide (for TA100) were used in experiments without without metabolic activation (-S9), while 2-AF (2-aminofluorene) was used for TA98 and TA100 strains with metabolic activation system (+S9). Freshly prepared minimal agar, top agar and overnight grown bacterial culture from a single colony (density of 1-2x 109 CFU/ml) were used in all the experiments. Dilutions of extract (100-2500 µg/0.1ml) were made in DMSO (Dimethyl sulfoxide). Non toxic concentrations of mutagens were used i.e. NPD (20 µg/0.1 ml), sodium azide (20 µg/0.1 ml), 2-AF (2.5 µg/0.1 ml). Spontaneous reversion frequency of bacterial strains was checked for every experiment. Extract was also evaluated for toxicity against all the mutagens used in the present study. Experiments were conducted in co-incubation and pre-incubation modes with and without S9. All the experiments were conducted in triplicates and percent inhibition of mutagenic activity was calculated as follows:

The inhibitory activity of aqueous peel extract was expressed as:

Inhibitory activity (%) = [(a-b) / (a-c)]* 100

Where ‘a’ is the number of histidine revertants induced by mutagen alone (positive control), ‘b’ is the number of histidine revertants induced by mutagen in the presence of extract, and ‘c’ is the number of histidine revertants induced in the presence of extract alone and solvent (negative control).

RESULTS AND DISCUSSION

Aqueous peel extract exhibited strong antimutagenic activity against NPD and 2-AF in TA98 strain of *Salmonella typhimurium* as shown in Table 1. Extract showed less effect in co-incubation mode as it showed maximum inhibition of 27.67% in co-incubation, while in pre-incubation mode it showed 66.38% inhibition against NPD. The extract exhibited 74.31% and 73.67% inhibition against 2-AF in co-incubation and pre-incubation modes respectively in TA98 strain of *Salmonella typhimurium* (Graph 1). In TA100 strain of *Salmonella typhimurium*, aqueous peel extract exhibited 37.78% and 66.38% inhibition in S9 independent mode in co-incubation and pre-incubation modes respectively, while in S9 dependent mode, the inhibition was found to be 69.69% and 70.93% in co-incubation and pre-incubation modes respectively at highest tested concentration (Table 2 and Graph 2). All the results were found statistically significant in both one-way and two-way ANOVA (Table 1 and 2). The logistic regression between percentage inhibition of mutagenic activity of aqueous peel extract in sigmoid model was found to be significant at $p \leq 0.001$ (Graph 3-10). The extract showed more effect in pre-incubation mode in TA98 strain as compared to co-incubation mode. This enhanced effect in pre-incubation mode suggests that compounds in extract got a better chance to react with mutagens in this short period of incubation. The effect was found to be effective in reducing the number of frameshift mutations induced by 2-AF on *Salmonella typhimurium* TA98 as well as base pair substitutions on *Salmonella typhimurium* TA100. The extracts showed significant enhancement in inhibition of mutagenic activity when metabolized in the presence of S9. Similar experiment was conducted by Geetha and Santhy[8] on orange peels and found significant antimutagenic effect against sodium azide and daunomycin in TA98 and TA100 of *Salmonella typhimurium.* Ames assay is a preliminary test for the screening of plant extracts for mutagenicity/antimutagenicity and extracts show their effects by different mechanisms. Therefore, further studies are required to check antimutagenicity of aqueous peel extract in other standard *in vitro* and *in vivo* assays. Since, many studies indicate that cancer is the second leading cause of death[9] in the world therefore antimutagenic and anticancer compounds of natural origin should be searched out globally.

Table 1
Antimutagenic activity of aqueous peel extract against NPD and 2-AF in TA98 strain of Salmonella typhimurium

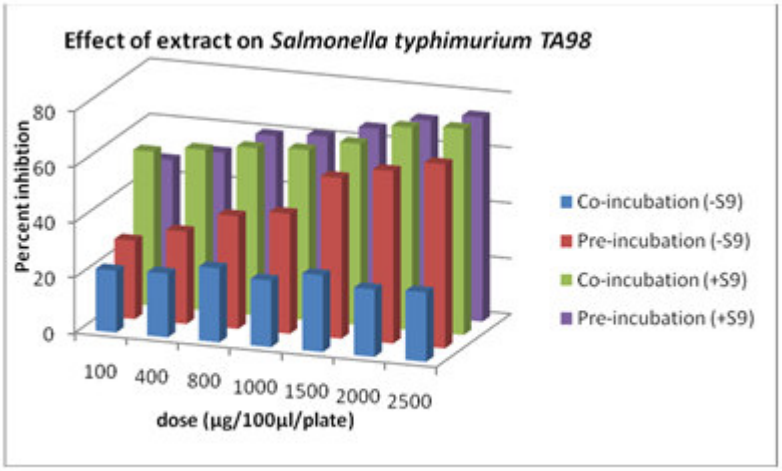
Type of treatment	Concentration (µg/100µl/plate)	*Salmonella typhimurium* (TA98)			
		Without metabolic system (-S9)		With metabolic system (+S9)	
		Number of revertants/plate	Percent inhibition	Number of revertants/plate	Percent inhibition
Spontaneous		26.33±1.53		23.67±1.15	
Positivecontrol					
NPD	**20**	954.00±24.25			
2-AF	**20**	-		2370.67±18.23	
Negative control	**100**	22.33±1.15		23.67±1.15	
	400	23.00±3.00		28.00±2.00	
	800	26.67±3.06		25.33±0.58	
	1000	24.00±1.00		23.00±1.00	
	1500	27.67±2.08		23.00±1.73	
	2000	24.33±0.58		24.33±2.08	
	2500	25.00±1.73		23.33±0.58	
Co-incubation	**100**	858.00±15.13	22.33±1.15	1066.00±44.54	55.59±1.92
	400	797.00±11.79	23.00±3.00	1011.67±25.32	58.01±1.13
	800	771.33±8.74	26.67±3.06	954.33±7.51	60.39±0.33
	1000	718.67±13.50	24.00±1.00	929.00±14.73	61.41±0.62
	1500	695.33±11.24	27.67±2.08	835.00±6.56	65.41±0.29
	2000	657.33±14.98	24.33±0.58	658.00±9.00	72.99±0.34

	2500	603.00±7.94	25.00±1.73	626.33±9.71	74.31±0.43
Pre-incubation	**100**	690.67±19.86	28.27±2.16	1250.33±19.14	47.73±0.80
	400	644.00±11.79	33.30±1.19	1149.33±17.50	52.13±0.79
	800	577.00±8.72	40.65±0.91	963.00±8.54	60.02±0.36
	1000	551.67±9.50	43.26±1.00	928.00±7.81	61.45±0.35
	1500	417.00±14.00	57.97±1.41	821.67±11.06	65.98±0.52
	2000	376.00±18.00	62.17±1.90	710.67±14.98	70.75±0.67
	2500	337.33±7.51	66.38±0.92	641.33±15.31	73.67±0.65
One-way ANOVA					
Positive control and Co-incubation		F(7,16)= 188.95***; HSD=40.36		F(7,16)=2135.80***; HSD=58.74	
Positive control and Pre-incubation		F(7,16)=520.61***; HSD=43.18		F(7,16)=4240.37***; HSD=41.48	
Two-way ANOVA					
Co-incubation and Pre-incubation					
Treatment		F(1,28)=2941.35***		F(1,28)=98.20***	
Concentration		F(6,28)=448.94***		F(6,28)=701.97***	
Treatment x Concentration		F(6,28)=29.96***		F(6,28)=27.29***	
HSD value		HSD=38.491		HSD=53.677	

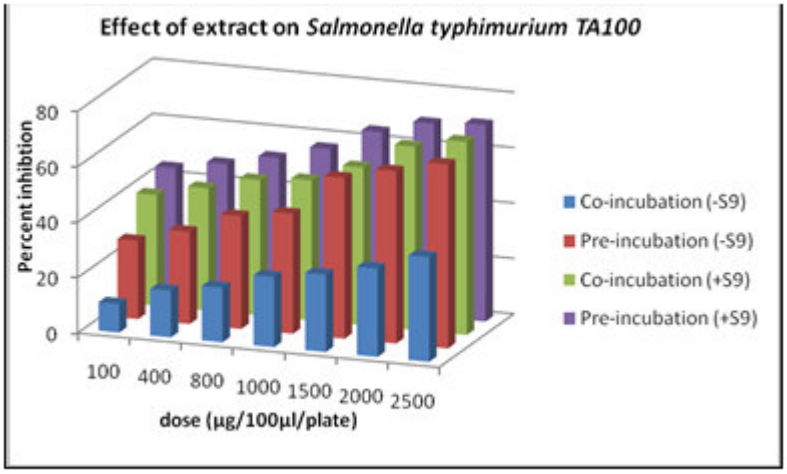
Table 2
Antimutagenic activity of aqueous peel extract against sodium azide and 2-AF in TA100 strain of Salmonella typhimurium

Type of treatment	**Concentration (µg/100µl/plate)**	***Salmonella typhimurium* (TA100)**			
		Without metabolic system (-S9)		**With metabolic system (-S9)**	
		Number of revertants/plate			**Number of revertants/plate**
Spontaneous		257.67±9.50		254.67±6.66	
Positive control					
Sodium azide	**2.5**	1973.67±15.37			
2-AF	**20**	-		2593.33±42.62	
Negative control	**100**	223.00±12.00		225.67±5.69	
	400	244.33±7.02		245.33±18.61	
	800	241.33±19.86		236.00±10.54	
	1000	255.00±18.52		238.00±10.54	
	1500	259.67±33.83		262.00±13.11	
	2000	243.00±18.36		236.33±13.01	
	2500	243.33±7.37		236.67±10.60	
Co-incubation	**100**	1437.33±17.62	10.30±1.62	1646.00±16.64	40.01±0.70
	400	1355.33±8.50	16.86±1.25	1558.00±18.33	44.10±1.07
	800	1256.67±21.13	19.70±0.89	1441.67±14.19	48.85±0.39
	1000	1229.00±20.66	25.31±1.46	1402.67±16.77	50.55±0.91
	1500	1190.00±26.51	27.93±1.27	1266.67±15.18	56.91±0.57
	2000	1138.33±12.34	31.91±1.63	1033.67±8.33	66.17±0.67
	2500	1086.00±20.66	37.78±0.92	951.00±17.69	69.69±0.46
Pre-incubation	**100**	1343.33±14.36	28.27±2.16	1531.33±3.06	44.85±0.11
	400	1245.33±22.55	33.30±1.19	1464.33±10.12	48.08±0.19
	800	1039.33±12.86	40.65±0.91	1367.67±5.13	51.99±0.20
	1000	1030.33±17.62	43.26±1.00	1251.00±17.00	56.99±0.65
	1500	962.00±17.06	57.97±1.41	1081.67±12.66	64.84±0.58
	2000	930.33±11.15	62.17±1.90	951.67±14.57	69.65±0.70
	2500	889.67±15.63	66.38±0.92	921.67±12.01	70.93±0.39
One-way ANOVA					
Positive control and Co-incubation		F(7,16)=689.30***; HSD=52.69		F(7,16)=1755.61***; HSD=59.38	
Positive control and Pre-incubation		F(7,16)=1474.26***; HSD=45.69		F(7,16)=2483.73***; HSD=52.55	
Two-way ANOVA					
Co-incubation and Pre-incubation					
Treatment		F(1,28)=1072.16***		F(1,28)=603.30***	
Concentration		F(6,28)=401.63***		F(6,28)=1996.40***	
Treatment x Concentration		F(6,28)=13.87***		F(6,28)=21.13***	
HSD value		HSD=52.979		HSD=41.188	

Graph 1-2
Effect of aqueous peel extract in co-incubation and pre-incubation modes



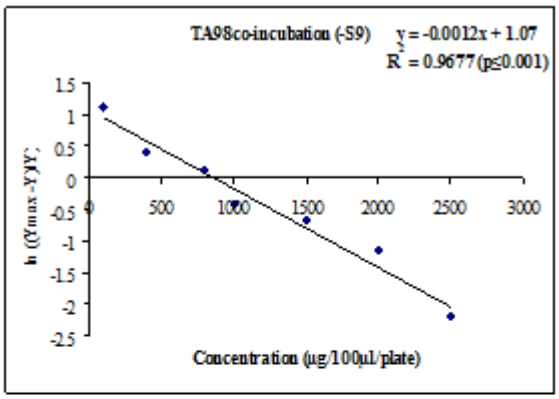
Graph 1



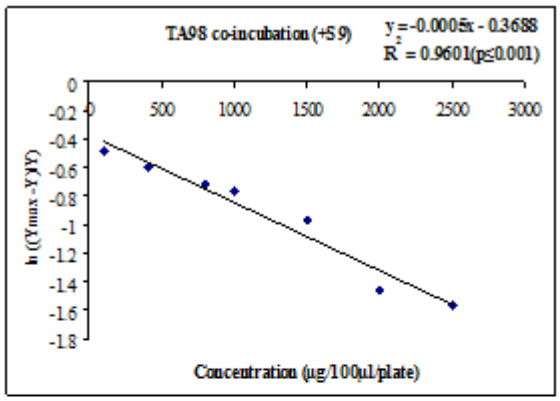
Graph 2

Graph 3-10

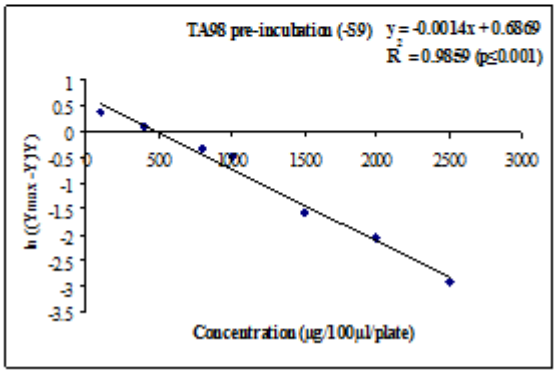
Logistic regression between percentage inhibition of mutagenic activity of mutagens in TA98 and TA100 tester strains of S. typhimurium and concentration of aqueous peel extract of C. borivilianum using sigmoid model



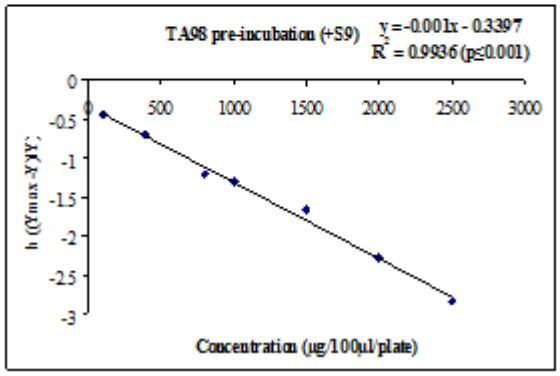
Graph 3



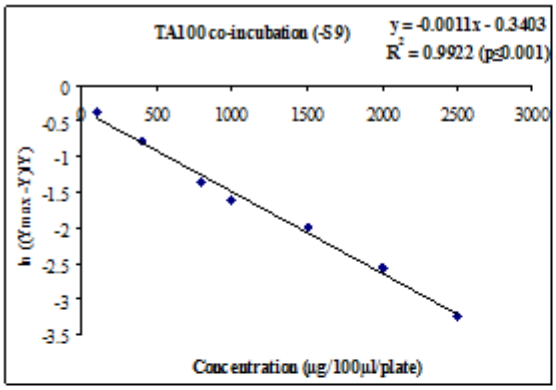
Graph 4



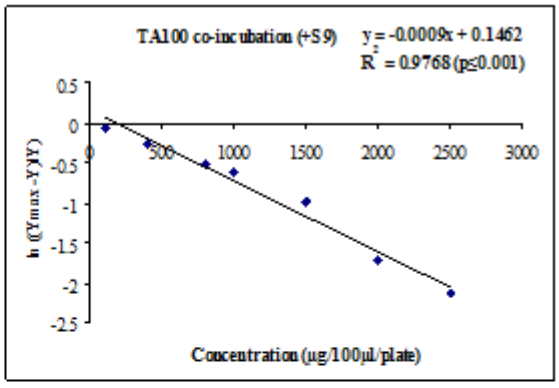
Graph 5



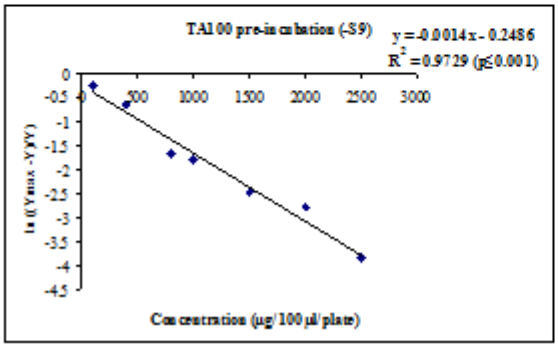
Graph 6



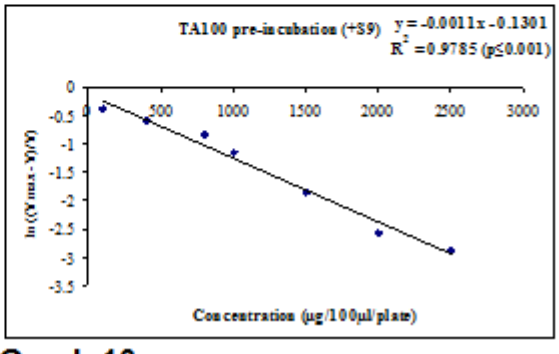
Graph 7



Graph 8



Graph 9



Graph 10

CONCLUSION

Aqueous peel extract can be used as a source of antimutagenic compounds. However, further studies are needed to analyze the bioactive components of peels and the mechanisms involved in the inhibitory effects against mutagens.

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