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SCREENING OF IN-VITRO ANTI-MUTAGENIC ACTIVITY OF SELECTED PLANTS

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ABSTRACT

Any agent, which increases DNA damage or cell proliferation, can cause increased rate of mutation also increase the probability of cancer. A mutagen is considered an agent capable of destroying the integrity of hereditary mechanism of the cell or organism. Majority of known cancer causing agents are mutagens. The rate of tumor evolution and progression is accelerated by mutagenic agents. Mutation is now a day's increase in human being. Here screening of in vitro anti-mutagenic activity of selected plants *Spheranthus indicus*, *Asteracantha longifolia*, *Jateorhiza palmata*, *Mucuna Pruriens*, *Tecomella undulate*, *Picrorhiza kurroa*, *Grewia tiliifolia*, *Myristica fragrans*, *Oroxylum indicum*, *Gymnosporia montana* were investigated. The activity was assayed by Ames Salmonella mutagenicity test using histidine mutants of Salmonella typhimurium tester strains, MTCC 98, MTCC 1251 and MTCC 1252. The hydroalcoholic extract of the plants significantly inhibited ($P < 0.001$) the *in vitro* by direct mutagens sodium azide (NaN_3), 4-nitro-o-phenylenediamine (NPD), and indirect mutagens benzo[a]pyrene (B[a]P) 2-aminoflourene(2-AF) induced his revertants in adose dependent manner. The results indicated that the hydroalcoholic extract of *Spheranthus indicus* occurring in India possessed significant antimutagenic activity.

Keywords: In-vitro, Anti-mutagenic, Salmonella typhimurium, mutagens

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INTRODUCTION

In India, many forms of alternative medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine. Ayurveda and Kabiraji (herbal medicine) are two important forms of alternative medicine that is widely available in India¹. Herbal medicine involves the use of plants for medicinal purposes. The term “Herb” includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs². About 80 % of individuals from developed countries use traditional medicine, which has compound derived from medicinal plants. The quest for plants with medicinal properties continues to receive attention as scientists are in need of plants, particularly of ethno botanical significance for a complete range of biological activities, which ranges from antibiotic to anticancerous. Herbal plants are useful against cancer Alteration in genetic material results in mutation. In 1901, Hugo de Vries advanced the hypothesis of mutation, as abrupt spontaneous origin of new character. Any agent, which increases DNA damage or cell proliferation, can cause increased rate of mutation also. Mutation may alter the regulatory control; such as mutation with a somatic cell may results in uncontrolled cell division leading to cancer.

A mutagen is considered an agent capable of destroying the integrity of hereditary mechanism of the cell or organism. Any substance causing increased mutation can also increase the probability of cancer. Vast majority of cancers are initiated by genetic changes. A carcinogen is a chemical capable of increasing the incidence of cancer in any species. The carcinogenesis appears to be linked with mutagenesis. Majority of known cancer causing agents are mutagens. The rate of tumor evolution and progression is accelerated by mutagenic agents. Human body is continuously and unavoidably exposed to a plethora of structurally diverse chemicals (polyaromatic hydrocarbon, aromatic amines and heterocyclic amine) which have established carcinogenic activity in animal model and / or mutagenic activity in short term tests. A characteristic of the above major classes of chemical carcinogens namely poly-aromatic hydrocarbons, heterocyclic amines and aromatic amines is that in order to express their genotoxicity and carcinogenicity, they must be metabolized to reactive intermediates that have the capacity to interact covalently with DNA. Damage to DNA is likely to be a major cause of cancer and other diseases. Pollution of the environment by manmade chemical toxicants is

difficult to control for obvious reasons. Hopefully the genotoxic effects of toxicants can be minimized by modulation of the physiological detoxification. Many naturally occurring compounds with antioxidant activity are known to protect cellular components from oxidative damage and prevent diseases³. A number of such compounds can activate the phase 2 detoxification enzymes, which can remove the toxic elements from our system. Exposure to such phytochemical is therefore beneficial to human health. A considerable emphasis is being placed on the use of dietary to human health. A considerable emphasis is being placed on the use of dietary constituents to prevent mutagenesis and carcinogenesis due to their relative non-toxic properties. Mutations are the cause of innate metabolic defects in cellular systems, triggering morbidity and mortality in living organisms. A plethora of synthetic and natural substances, apart from various genotoxic physical and biological agents are known to act as mutagenic, co-carcinogenic and/or carcinogenic agents. There is increasing evidence that mutation in somatic cells are not only involved in the carcinogenesis but can also cause genetic disorders like atherosclerosis, heart diseases and several other degenerative disorders⁴. Since, the mutagens are involved in the initiation and promotion of several human diseases, including cancer, the significance of novel bioactive phytochemicals in counteracting these pro-mutagenic and carcinogenic effects is now gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are referred to as antimutagens⁵. The group of chemicals that cause cancer in man and animals are collectively referred to as carcinogens. Environmental pollution is associated with increased risk of cancer. Prevention of cancer and other mutation related diseases can be pursued by avoiding exposure to recognized carcinogens or mutagens, by favoring the intake of protective factors and by fortifying physiological defense mechanism. Moreover, there is an increasing awareness that certain naturally occurring substances in plants and other source have protective effects against environmental mutagens or carcinogens and also endogenous mutagens. Hence, research work related to the discovery, characterization and use of antimutagenic agents is receiving considerable attention. A large number of experimental reports have begun to appear in the scientific literature, wherein increasingly more natural antimutagens have been identified, isolated and found to possess significant mutation chemoprevention properties. Therefore, screening of *in vitro* anti-mutagenic activity of some selected plants *Spheranthus indicus*, *Asteracantha longifolia*, *Jateorhiza palmata*, *Mucuna pruriens*, *Tecomella undulate*, *Picrorhiza kurroa*, *Grewia tiliifolia*, *Myristica fragrans*, *Oroxylum indicum*, *Gymnosporia montana* were investigated.

MATERIAL AND METHODS

Experimental material

Here screening of in vitro anti-mutagenic activity of some selected plants are *Spheranthus indicus*, *Asteracantha longifolia*, *Jateorhiza palmata*, *Mucuna Pruriens*, *Tecomella undulate*, *Picrorhiza kurroa*, *Grewia tiliifolia*, *Myristica fragrans*, *Oroxylum indicum*, *Gymnosporia montana*. Crude plants were authenticated in the department of pharmacognosy, by morphological characters and were authenticated by taxonomist also. The voucher specimens were deposited in K.B. Institute of pharmaceutical Education and Research, Gandhinagar.

Preparation of different extracts

The selected plants *Spheranthus indicus*, *Asteracantha longifolia*, *Jateorhiza palmata*, *Mucuna Pruriens*, *Tecomella undulate*, *Picrorhiza kurroa*, *Grewia tiliifolia*, *Myristica fragrans*, *Oroxylum indicum*, *Gymnosporia montana* were dried under sunlight. Dried powdered passed through sieve of 60 mesh (#) size and stored in airtight containers. The powdered materials (20 g) were extracted with hot methanol: water (70:30) at 70–80⁰C twice. Hydroalcoholic extracts were pooled, concentrated and evaporated under vacuum. The extracts thus obtained were used for the experiments.

Chemicals

Glucose-6-phosphate, l-histidine, d-biotin were procured from Sisco Research Laboratories, Mumbai, India. Benzo [α] pyrene (B[a]P), 4-nitro-*o*-phenylene diamine (NPD), Sodium azide (NaN₃) was procured from Hi-Media, Mumbai, India. All other chemicals employed in the studies were of analytical grade (AR grade).

Bacterial strain

Histidine requiring strains of *Salmonella typhimurium* MTCC 98, MTCC1251 and MTCC 1252 were obtained from MTCC, Chandigarh. They were incubated in nutrient broth for 12 h and frozen permanents were prepared by freezing at –70⁰C in the presence of 9% dimethyl sulfoxide (DMSO). Fresh cultures were prepared by inoculating 40 μ l of frozen permanents in 5ml of nutrient broth and incubated for 12 h at 37⁰C. These cultures were used for the experiments.

Preparation of mutagens

All of the chemical mutagens were dissolved in dimethyl sulfoxide (DMSO) except sodium azide, which was dissolved in water.

Animals: Wistar albino rat (KBIPER, Gandhinagar, India) was used for the current study. It was housed in well ventilated cage and fed with standard pelleted diet and kept at air-controlled

room. Animal experiment was conducted according to guidelines and following the approval of the Institutional Animal Ethical Committee.

Determination of in vitro antimutagenicity

A) Direct acting mutagens

Antimutagenicity of *different* extracts against direct acting mutagens was determined according to the methods of Maron and Ames⁶. For this 2 ml of top agar containing 0.2 ml of 0.5mM histidine–biotin was mixed with mutagens (NaN₃, NPd). At a concentration of 1, 2, 3 mg/plate of *Spheranthus indicus*, *Asteracantha longifolia*, *Jateorhiza palmata*, *Mucuna Pruriens*, *Tecomella undulate*, *Picrorhiza kurroa*, *Grewia tiliifolia*, *Myristica fragrans*, *Oroxylum indicum*, *Gymnosporia montana* extract were dissolved in distilled water and 0.1 ml freshly grown *typhimurium* culture (1×10⁹ cells/ml approximately) were poured in to minimal agar plates and incubated at 37⁰C for 48 h. After the incubation, the revertants colonies were counted by using a colony counter.

B) Mutagens requiring activation

Antimutagenic assay against mutagen that require metabolic activation Benz[a]pyrene (B[a] P) and 2-Aminofluorene (2-AF) was carried out as follows. Liver microsomal fraction (S9) was prepared from rat liver. The rat was treated with 0.1% Phenobarbitone in drinking water for 4 days⁷. After overnight fasting the animal was killed by decapitation, the liver was removed and the homogenate was prepared aseptically³. The activation mixture was prepared by mixing 50 µl of the S9 fraction, containing 0.25 ml phosphate buffer (0.2 M, pH 7.4), 20µl NADP (0.1 M), 2.5µl glucose-6-phosphate (1 M) and 10µl of 1.65M MgCl₂– 0.4M KCl and various concentration of extract (3, 2, or 1 mg) mixed with the mutagens at a given concentration poured in to minimal agar plates and incubated for 48 h at 37⁰C. After incubation, number of revertants was counted using a colony counter. Toxicity of different extracts, if any, against bacterial strains was determined by incubating various concentrations of different extracts with cultures of different tester strains of *Salmonella* for 48 h and checking the number of revertants and background lawn. Percent inhibition of mutagenicity was determined as per the following formula:

$$\text{Inhibition (\%)} \text{ of mutagenicity} = \frac{(R1 - SR) - (R2 - SR)}{(R1 - SR)} \times 100$$

Where, R1 is the number of revertants without different extract, R2 the number of revertants with different extract and SR is the spontaneous revertants. The experiments were carried out in triplicate.

Statistical analysis

The results were expressed as Mean \pm SEM. The significance of difference between mean values for the various extracts was tested.

RESULTS AND DISCUSSION:

Determination of in vitro antimutagenicity

A) Direct acting mutagens

Antimutagenic activity of selected plant extract against sodium azide and 4-nitro-O-phenylene diamine (NPD) as shown in Table 1 and 2 respectively.

Table : 1 Antimutagenic activity of selected plant extracts against sodium azide.

Name of the plant	Concentration (mg/plate)	Average number of revertants/plate		% Inhibition	
		MTCC 1251 mean \pm S.D	MTCC 1252 mean \pm S.D	MTCC 1251	MTCC 1252
Control	0.0025	1234.6 \pm 12.2	435.3 \pm 19.5	-----	-----
<i>Spheranthus indicus</i>	1	0632.3 \pm 18.7	156.0 \pm 15.2	52.81	75.14
	2	0535.6 \pm 11.5	073.3 \pm 11.5	61.29	97.39
	3	0441.6 \pm 10.0	066.6 \pm 17.5	69.53	99.19
<i>Asteracantha longifolia</i>	1	0883.6 \pm 10.5	156.3 \pm 13.2	30.77	75.06
	2	0724.0 \pm 15.0	120.6 \pm 14.1	44.77	84.66
	3	0528.3 \pm 24.0	067.6 \pm 16.8	61.93	98.92
<i>Jateorhiza palmata</i>	1	0825.3 \pm 12.0	234.3 \pm 09.7	35.89	54.16
	2	0674.3 \pm 13.6	206.3 \pm 09.6	49.13	61.60
	3	0454.3 \pm 07.2	256.3 \pm 18.5	68.42	48.15
<i>Mucuna pruriens</i>	1	0595.3 \pm 12.3	095.6 \pm 12.3	56.05	91.39
	2	0516.6 \pm 09.7	087.3 \pm 09.0	62.96	93.62
	3	0212.6 \pm 17.3	078.6 \pm 04.5	89.61	95.96
<i>Tecomella undulate</i>	1	0851.0 \pm 17.0	275.6 \pm 10.6	33.63	42.96
	2	0759.3 \pm 30.3	241.0 \pm 11.3	41.67	52.27
	3	0548.6 \pm 12.2	185.0 \pm 10.1	60.15	67.33
<i>Picrorhiza kurroa</i>	1	1194.0 \pm 08.1	120.3 \pm 17.1	03.56	84.74
	2	1140.1 \pm 14.1	096.1 \pm 13.0	08.28	91.25
	3	1109.0 \pm 14.0	087.3 \pm 04.1	11.01	93.62
<i>Grewia tiliifolia</i>	1	0436.3 \pm 11.5	163.6 \pm 16.9	70.00	73.01
	2	0231.0 \pm 09.8	128.3 \pm 12.4	88.00	82.59
	3	0142.0 \pm 14.7	084.0 \pm 11.1	95.80	94.51
<i>Myristica fragrans</i>	1	0559.6 \pm 15.6	279.6 \pm 14.5	59.18	41.88
	2	0368.6 \pm 25.6	252.6 \pm 16.0	75.93	49.31
	3	0145.0 \pm 12.5	248.3 \pm 15.2	95.54	50.30
<i>Oroxylum indicum</i>	1	0953.6 \pm 10.6	164.3 \pm 15.5	24.64	72.90
	2	0843.3 \pm 21.0	143.6 \pm 12.5	34.31	78.47
	3	0756.3 \pm 18.5	087.0 \pm 11.5	41.94	93.70

<i>Gymnosporia montana</i>	1	0340.6±16.1	129.6±21.7	78.39	82.24
	2	0262.0±17.6	124.0±30.1	85.28	83.75
	3	0159.3±13.3	084.3±13.6	94.29	94.43
SR		0094.2±06.5	063.6±12.3	-----	-----

Value are mean ± S.D (n=3).

Table : 2 Antimutagenic activity of selected plant extracts against 4-nitro-O-phenylene diamine.

Name of the plant	Concentration (mg/plate)	Average number of revertants/plate		% Inhibition	
		MTCC 98 mean ± S.D	MTCC 1251 mean ± S.D	MTC C 98	MTCC 1251
Control	0.0025	1268.3±25.1	1351.6±33.0	-----	-----
<i>Spheranthus indicus</i>	1	0961.3±18.5	0869.6±25.5	26.61	38.19
	2	0760.3±36.5	0565.3±33.5	44.03	59.05
	3	0437.6±34.0	0469.3±18.6	72.00	67.31
<i>Asteracantha longifolia</i>	1	1155.3±27.2	0970.0±15.8	09.79	24.23
	2	1062.6±32.1	0862.6±22.3	17.89	33.47
	3	0957.3±15.0	0547.6±14.5	26.95	60.57
<i>Jateorhiza palmata</i>	1	0959.0±25.6	1151.0±33.6	26.80	08.65
	2	0655.3±33.0	1060.6±31.9	53.13	16.43
	3	0477.6±16.2	0961.6±16.5	68.43	24.95
<i>Mucuna pruriens</i>	1	0862.3±36.0	1060.6±31.0	35.19	16.43
	2	0662.6±22.3	0854.3±19.7	52.50	34.18
	3	0452.3±38.6	0555.6±27.7	70.72	59.89
<i>Tecomella undulate</i>	1	0960.3±18.0	0764.3±25.0	26.69	41.93
	2	0869.6±22.4	0657.3±15.6	34.55	51.16
	3	0741.0±29.2	0244.0±32.6	45.70	86.70
<i>Picrorhiza kurroa</i>	1	0966.0±22.2	0859.6±16.6	26.20	33.73
	2	0831.0±31.7	0766.3±37.7	37.90	41.76
	3	0744.3±41.5	0549.0±43.2	45.41	60.45
<i>Grewia tiliifolia</i>	1	0451.3±41.0	0953.0±28.9	70.81	25.69
	2	0346.6±46.5	0833.6±34.0	79.89	35.96
	3	0153.0±38.7	0542.6±32.5	96.67	61.01
<i>Myristica fragrans</i>	1	1109.3±78.1	1042.0±40.9	13.80	18.03
	2	1043.3±48.0	0848.0±28.4	19.50	34.73
	3	0951.3±17.3	1153.3±37.8	27.47	08.45
<i>Oroxylum indicum</i>	1	0950.3±33.5	1048.3±49.0	27.56	17.52
	2	0752.0±44.9	0734.3±44.4	44.75	44.51
	3	0545.0±39.6	0644.3±36.8	62.69	52.29
<i>Gymnosporia montana</i>	1	1167.6±32.6	1060.0±32.1	08.72	16.48
	2	1055.0±47.2	0651.0±42.3	18.48	51.68
	3	0859.6±32.5	0269.3±31.8	35.42	84.52
SR		0114.6±54.2	0089.5±42.3	-----	-----

Value are mean ± S.D (n=3).

Hydroalcoholic extracts of *Grewia tiliifolia*, *Myristica fragrans*, *Gymnosporia Montana*, *Spheranthus indicus*, *Asteracantha longifolia*, *Mucuna Pruriens*, *Picrorhiza kurroa*, *Oroxylum indicum* showed mutagenic inhibition by direct acting mutagen of sodium azide against both the strains MTCC 1251 and MTCC 1252.

Hydroalcoholic extracts of *Grewia tiliifolia*, *Myristica fragrans*, *Gymnosporia Montana*, *Spheranthus indicus*, *Asteracantha longifolia*, *Mucuna pruriens*, *Picrorhiza kurroa*, *Oroxylum indicum* showed mutagenic inhibition by direct acting mutagen of 4-nitro-O-phenylene diamine (NPD) against both the strains MTCC 98 and MTCC 1251.

B) Mutagens requiring activation

Antimutagenic activity of selected plant extract against Benzo[α]pyrene and 2-Aminofluorene as shown in Table 3 and 4 respectively.

Hydroalcoholic extracts of *Gymnosporia montana*, *Myristica fragrans*, *Mucuna pruriens*, *Spheranthus indicus* showed mutagenic inhibition by direct acting mutagen of Benzo[α] pyrene against both the strains MTCC 1251 and MTCC 1252.

Hydroalcoholic extracts of *Jateorhiza palmate*, *Gymnosporia Montana* showed mutagenic inhibition by direct acting mutagen of 2-Aminofluorene against both the strains MTCC 1251 and MTCC 1252.

A mutagen is considered as an agent of destroying the integrity of hereditary mechanism of cell or organism. Any substance that cause increased mutation can also increase the probability of cancer. The rate of tumor evolution and progression is accelerated by mutagenic agents. Hence, peroxidation of mutation is paramount importance for the prevention of cancer.

Considerable attention has been focused on the role of dietary supplements and their constituents as chemopreventive agents in recent years⁸. Dietary interactions that decrease the mutagenic load and abnormal biological responses appear to be one of the plausible approaches for cancer prevention. Significant correlations have been observed between the carcinogenicity of a series of polycyclic aromatic hydrocarbons (PAH) and their covalent binding to mouse epidermal DNA^{9, 10, 11}. Based on extensive evidence accumulated in the last two decades, it is believed that PAH must be metabolically activated to electrophilic intermediates, which can bind to DNA and exert its carcinogenic effects¹². B[a] P is metabolized by mixed function oxidase (MFO) of rat liver to active intermediate benzo[a]pyrene-7, 8-diol, 9, 10-epoxide [BPDB]¹³. These can attack cellular macromolecules like DNA, RNA, proteins, membranes, etc., and cause dysfunction and damage. The reactive oxygen species are important as direct and indirect initiators as well as

Table : 3 Antimutagenic activity of selected plant extracts against Benzo[α]pyrene.

Name of the plant	Concentration (mg/plate)	Average number of revertants/plate		% Inhibition	
		MTCC 1251 mean \pm S.D	MTCC 1252 mean \pm S.D	MTCC 1251	MTCC 1252
Control	0.0025	542.3 \pm 38.5	750.3 \pm 30.0	-----	-----
<i>Spheranthus indicus</i>	1	364.3 \pm 27.6	225.3 \pm 21.1	37.88	81.01
	2	247.6 \pm 38.4	167.6 \pm 30.7	62.72	89.92
	3	213.3 \pm 25.8	156.0 \pm 38.3	70.02	91.71
<i>Asteracantha longifolia</i>	1	468.0 \pm 32.0	432.3 \pm 20.6	15.81	49.07
	2	348.6 \pm 38.3	362.6 \pm 23.2	41.23	59.83
	3	275.3 \pm 26.8	241.0 \pm 34.3	56.83	78.59
<i>Jateorhiza palmate</i>	1	449.3 \pm 43.3	448.6 \pm 42.0	19.79	46.55
	2	434.0 \pm 39.6	345.3 \pm 43.5	23.05	62.50
	3	348.0 \pm 40.1	244.6 \pm 32.5	41.35	78.04
<i>Mucuna Pruriens</i>	1	522.0 \pm 20.5	354.6 \pm 46.8	04.32	61.06
	2	459.6 \pm 16.8	244.0 \pm 41.5	17.60	78.13
	3	356.6 \pm 32.0	147.6 \pm 40.6	39.52	93.00
<i>Tecomella undulate</i>	1	206.6 \pm 20.5	370.0 \pm 33.6	71.45	58.68
	2	155.0 \pm 43.5	249.0 \pm 38.1	72.43	77.36
	3	125.3 \pm 43.2	208.0 \pm 18.0	88.82	83.68
<i>Picrorhiza Kurroa</i>	1	534.0 \pm 38.3	353.3 \pm 42.7	01.76	61.26
	2	437.6 \pm 43.2	258.3 \pm 36.5	22.28	75.92
	3	429.6 \pm 23.2	205.0 \pm 18.3	23.98	84.15
<i>Grewia Tiliifolia</i>	1	523.3 \pm 22.5	705.6 \pm 37.6	04.04	06.89
	2	436.0 \pm 37.7	629.0 \pm 22.6	22.62	18.71
	3	414.6 \pm 36.6	610.0 \pm 30.6	27.18	21.65
<i>Myristica fragrans</i>	1	341.3 \pm 36.6	358.6 \pm 41.0	42.78	60.44
	2	256.3 \pm 42.7	240.6 \pm 23.7	60.87	78.65
	3	154.0 \pm 30.6	146.6 \pm 42.0	82.65	93.16
<i>Oroxylum indicum</i>	1	355.6 \pm 34.5	358.0 \pm 37.0	39.74	60.54
	2	345.3 \pm 40.5	251.3 \pm 42.0	41.93	77.00
	3	334.6 \pm 28.9	260.0 \pm 26.8	44.21	75.66
<i>Gymnosporia montana</i>	1	256.3 \pm 28.0	458.3 \pm 42.3	60.87	45.06
	2	157.6 \pm 30.7	350.6 \pm 37.1	81.88	61.68
	3	086.5 \pm 11.7	247.0 \pm 38.0	97.02	77.66
SR		72.5 \pm 22.5	102.3 \pm 37.2	-----	-----

Value are mean \pm S.D (n=3).

promoters of mutagenesis and carcinogenesis. They also increase the lipid peroxidation, which in turn alter the integrity of membrane bound enzymes¹⁴. The free radical scavenging efficiency of the extract thus might be playing an important role in the antimutagenic activity. Most cancers are thought to originate from single cell that has experienced an initial mutation. The rate of tumor evolution and progress is accelerated both by mutagenic agents, tumor initiators and by

tumor promoters that effect gene expression, stimulate cell proliferation and alter ecological balance of mutant and non-mutant cells. A large number of cancer causing agents are mutagens. Tumor progression can be correlated with mutations that activate specific oncogens and inactivate tumor suppressor genes.

Table : 4 Antimutagenic activity of selected plant extracts against 2-Aminofluorene.

Name of the Plant	Concentration (mg/plate)	Average number of revertants/plate		% Inhibition	
		MTCC 1251 mean \pm S.D	MTCC 1252 mean \pm S.D	MTCC 1251	MTCC 1252
Control	0.0025	937.7 \pm 29.2	845.3 \pm 24.3	-----	-----
<i>Spheranthus indicus</i>	1	461.0 \pm 35.0	541.0 \pm 31.1	54.63	40.26
	2	420.0 \pm 22.1	475.3 \pm 12.6	59.33	48.95
	3	255.6 \pm 43.3	332.6 \pm 37.8	78.17	67.83
<i>Asteracantha longifolia</i>	1	688.0 \pm 18.0	540.6 \pm 31.2	28.61	40.31
	2	575.1 \pm 27.1	525.3 \pm 15.2	41.55	42.33
	3	352.6 \pm 35.3	359.3 \pm 36.1	67.06	64.30
<i>Jateorhiza palmata</i>	1	458.6 \pm 41.6	371.6 \pm 18.5	54.91	62.67
	2	416.3 \pm 16.9	329.1 \pm 17.6	59.75	68.29
	3	140.6 \pm 33.7	125.3 \pm 20.8	91.35	95.26
<i>Mucuna pruriens</i>	1	269.3 \pm 32.8	540.6 \pm 26.5	76.60	40.31
	2	238.6 \pm 15.6	660.6 \pm 31.5	80.12	24.43
	3	168.0 \pm 23.8	566.0 \pm 20.0	88.21	36.95
<i>Tecomella undulate</i>	1	366.0 \pm 19.5	451.3 \pm 38.5	65.52	52.13
	2	269.6 \pm 22.8	368.6 \pm 15.5	76.56	63.07
	3	255.1 \pm 30.5	335.1 \pm 12.4	78.23	67.50
<i>Picrorhiza Kurroa</i>	1	558.1 \pm 34.8	648.0 \pm 41.1	43.50	26.10
	2	461.3 \pm 24.0	560.6 \pm 29.2	54.60	37.66
	3	268.6 \pm 18.7	377.6 \pm 20.2	76.68	61.88
<i>Grewia Tiliifolia</i>	1	461.3 \pm 26.5	553.3 \pm 28.7	54.60	38.63
	2	372.2 \pm 25.6	551.7 \pm 33.7	64.81	38.84
	3	266.3 \pm 22.6	638.6 \pm 28.0	76.95	27.34
<i>Myristica fragrans</i>	1	248.1 \pm 15.7	438.3 \pm 34.9	79.03	53.89
	2	162.6 \pm 16.6	377.1 \pm 20.5	88.83	61.80
	3	351.6 \pm 31.5	281.6 \pm 19.2	67.17	74.58
<i>Oroxylum indicum</i>	1	869.6 \pm 22.4	784.6 \pm 17.8	07.80	08.03
	2	842.3 \pm 31.5	729.6 \pm 16.6	10.93	15.30
	3	754.1 \pm 30.0	744.3 \pm 42.0	21.04	13.36
<i>Gymnosporia montana</i>	1	551.2 \pm 27.5	170.2 \pm 22.6	44.29	89.32
	2	469.6 \pm 24.5	144.6 \pm 18.5	53.65	92.70
	3	367.6 \pm 32.6	121.3 \pm 17.3	65.34	95.79
SR		065.2 \pm 24.1	089.5 \pm 23.6	-----	-----

Value are mean \pm S.D (n=3).

All the medicinal plants have been use in traditional system of medicine throughout the world. Since ancient times, Agents that are capable to inhibit mutagenicity might be able to interfere with process of carcinogenesis and tumor promotion. Chemoprevention aimed at inhibiting or delaying the onset of carcinogenesis is a recently growing area of cancer research. Several line of evidence indicates the relation between mutagenesis and carcinogenesis. Hence, the antimutagenic properties of some selected plants were studied.

In the present study, Hydroalcoholic extracts of *Spheranthus indicus* plant showed significantly inhibition of mutagenicity induced by both direct acting mutagens sodium azide 4-nitro o-phenylene diamine and mutagens that require metabolic activation (benzo [α] pyrene and 2-amino flourine) in a dose dependent manner. The conclusion was based on the number of revertants and background lawn. Hence, the activity is not consequence of the toxic effect of Hydroalcoholic extracts of *Spheranthus indicus* plant on bacterial colony. Therefore, the present investigations were able to explain the mechanism by which the extracts inhibit the mutagenicity induced by both direct and indirect acting mutagens. This is significantly effective in preventing mutagenic activity induced by both direct and indirect acting mutagens, indicating their potentials in chemoprevention. Thus, the findings suggested that the potential of the hydroalcoholic extract of *Spheranthus indicus* act as a chemopreventive agent.

CONCLUSION:

It can be concluded from the present study, that the hydroalcoholic extract of *Spheranthus indicus* occurring in India possessed significant antimutagenic activity.

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