Available online at www.scholarsresearchlibrary.com

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2011, 1 (4):27-34**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Evaluation of *invitro* antimutagenic activity *of Caralluma adscendens Roxb*. in bacterial reverse mutation assay

[1]Gowri S* and [2]Chinnaswamy P

[1]PG and Research Department of Biochemistry, Dr. N. G. P. Arts and Science College, Coimbatore
[2]Institute of Laboratory Medicine, Kovai Medical Centre and Hospital, Coimbatore

---

ABSTRACT

Caralluma adscendens Roxb. is a thick, succulent perennial herb growing natural in dry hills areas. It belongs to family Asclepiadaceae and locally known as "Kalli Mulaiyan". The species of Caralluma found in India are edible and form part of the traditional medicine structure of the country. The herb contains Saponin glycosides, Flavonoids, Megastigmane glycosides, sitosterols and bitter principles. In addition Caralluma species are usually used in treatment of rheumatism, diabetes, leprosy, antipyretic and antihelmintic, for tumor, fungal diseases, snake, scorpion bite and antinociceptive activity. Ames Salmonella histidine reversion assay was used in the current exploration to evaluate antimutagenic activity of ethanolic extract of Caralluma adscendens Roxb. in TA 1535, TA98 and TA100 strains of Salmonella typhimurium using direct (Sodium azide, Ethidium bromide, Hydroxyl amine) acting mutagens. Results of the study showed significant antimutagenicity against all the mutagens in TA 1535, TA98 and TA100 tester strains. The antimutagenicity of the extract observed in the present study implies chemopreventive pharmacological importance of Caralluma adscendens Roxb and encourages its use as a functional food.

Key words: Antimutagenicity, Sodium azide, Hydroxyl amine, Ethidium bromide Salmonella typhimurium, Ames assay.

INTRODUCTION

Cancer and other chronic diseases share some common pathogenic mechanisms, such as DNA damage, oxidative stress, and chronic inflammation. These diseases can be controlled by forestalling the exposure to well-acknowledged risk factors and to render the organism more

resistant to mutagens/carcinogens and/or to inhibit progression of the disease by administering chemopreventive agents [1].

Chemotherapy and surgery are standard methods for treatment of these diseases, although not been fully effective. Most of the anti-tumor drugs currently used in chemotherapy are toxic to normal cells and cause toxicity for immune cells. Therefore, the identification of new anti-cancer drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology [2].

Mutations are the cause of inborn errors of metabolism leading to morbidity and mortality in living organisms. Besides inherited metabolic disorders, a spectrum of age related human diseases, including cancer, are caused by mutations. Mutagenic agents may be synthetic or natural toxic substances. Since cancer has become the number one cause of death, much attention has been focused on the chemoprevention of cancer, with little success. However, less attention has been given to the substances in medicinal plants and herbal medicines that may serve to protect against chemical mutagens or carcinogens acting as initiators in the carcinogenic process [3].

The rich diversity of Indian medicinal plants have not yet systematically screened for antimutagenic activity. Many plant species are known to elicit antimutagenesis and thus have a full range of prospective applications in human healthcare. Even for populations which use herbs traditionally, encouraging the use of species with chemopreventive actions could be helpful as part of life expectancy improvement strategies: costs are significantly low, herbs have usually little or no toxicity during long-term oral administration and are relatively available at large scale. It has been suggested that regularly consuming anticarcinogens and antimutagens in the diet may be the most effective way of preventing human cancer and search for novel antimutagens acting in chemoprevention is a promising field in phytotherapy [4].

Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention and have no undesirable xenobiotic effects on living organisms. Natural antioxidants may reduce or inhibit the mutagenic potential of mutagens and carcinogens. The cellular mutability control by natural antimutagens can provide ways for preventing mutations that conceivably result in cancer as well as diseases caused by genotoxic agents [5, 6].

Antimutagenic properties elicited by plant species have a full range of prospective applications in human health. Herbal remedies and phytotherapy drugs, containing active principles are currently developed to protect against electrophile (e.g free radical) attack on DNA and its widespread outcomes such as ageing and cancer. The occurrence rate of cancer is increasing worldwide and the determination of chemopreventive or chemoprophylaxis compound is important in the effort to reduce the risk of cancer. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen; however, it is an indication of possible candidates for such purposes [7].

The Ames test was used in this study as it is widely used in the determination of possible gene mutations caused by extracts. A positive response in any single bacterial strain either with or

without metabolic activation is sufficient to designate a substance as an antimutagen. The objective of this study was to evaluate the antimutagenic potential of plant extract by studying the effects on three histidine requiring strains of *Salmonella typhimurium.* The assay acts by detecting back mutations in the His− operon (→His+) when growing *Salmonella typhimurium* bacteria in a His-poor medium [8].

Caralluma adscendens Roxb a genus of the family Asclepiadaceae is an important medicinal and widely distributed succulent plant found in dry regions of world. Ethnobotanically. It is being used to cure diabetes and fat accumulation or as vegetable in different regions of India. The species of this family have significant anti inflammatory and antitumor activity, anticancer, cytoprotective and antiulcer activity, antinociceptive, antioxidant, hypolipidemic, antihyperglycemic, antidiabetic, treating paralysis and joint pains, antipyretic. The phytochemical constituents of the herb are Pregnane glycosides, Flavone glycoside (Chemotaxonomic marker), Saponin glycoside and Megastamine glycoside etc [9].

MATERIALS AND METHODS

Plant material and extraction procedure

The plant material was collected in Coimbatore, South India, and dried at 50° C. Storage was done in brown paper bags to ensure adequate aeration while keeping the material in the dark. Dried plant material was ground to a powder and 10 g of it was extracted with ethanol at room temperature. The crude extracts were filtered (Whatman No. 1 filter paper) and solvents were evaporated at 60 ° C under reduced pressure. The extract was dried completely to remove all traces of solvent before testing.

Chemicals

Sodium azide NaN_3, Ethdium omide , Hydroxyl amine, Dimethyl sulfoxide, Histidine, biotin, Amphicillin,

Bacterial strains

Salmonella typhimurium strains TA100, TA98 and TA1535 which are histidine-requiring mutants and the genotypes of the test strains were checked routinely for their histidine requirement, deep rough (rfa) character, UV sensitivity (uvr B mutation) and presence of the R factor. They were stored at −80 °C. A fresh nutrient broth culture was grown to a density of 1-2 X 10^9 cells/ml for 12 hour at 37°C before each experiment.

Confirming genotypes of the *Salmonella* strains

Histidine Requirement

The histidine character of the tester strains was confirmed by indicating the histidine requirement for growth on selective agar plates such as histidine/biotin plate and biotin control plate. Biotin was also mandatory for all of the tester strains because of the UvrB deletion which extended through the bio-gene. Cotton swab was dipped in the 12 hour broth culture and a single sweep was made across the histidine/biotin plate. Then, the plates were incubated overnight at 37^{o}C and the growth was examined on the next day.

rfa Mutation :

Strains having the deep rough (rfa) character were tested for crystal violet sensitivity (Ames et al., 1973). 0.1 ml of fresh overnight culture of the tester strains (TA 98, TA 100 and TA 1535) was added to a tube containing 2 ml of molten agar at 45^{o}C. The top agar tubes were vortexed for 3 seconds at low speed and poured on nutrient agar plate without histidine and biotin. The plates were tilted and rotated for the even distribution of the top agar on the plates. The plates were placed in a leveled surface and allowed several times for agar to become firm. 10 µl of 1 mg/ml solution of crystal violet was pipetted to the center of sterile paper disc (1/4 inch) and discs were transferred to each of the inoculated plates using sterile forceps. The discs were lightly pressed with forceps to embed it slightly in the overlay. The plates were incubated at 37^{o}C and observed for crystal violet sensitivity.

UVrB Mutation:

The UVrB mutation was confirmed by demonstrating UV sensitivity in strains that contain this mutation (Ames et al., 1973). The R-factor strains TA 98, TA 100 and non R-factor strains TA 1535 were streaked in parallel strips with sterile swabs across the nutrient agar plate. A piece of cardboard was placed over the uncovered plate so that half of each of bacterial streak was covered. The plates were irradiated with a 15 W germicidal lamp approximately at a distance of 35 cm and were irradiated for 8 seconds. The irradiated plated were incubated at 37^{o}C for 12-24 hours.

R-Factor

The R-factor strains TA 98 and TA 100 were tested for the presence of the ampicillin resistance factor. To test for ampicillin resistance, the cultures were streaked across of an ampicillin plate using the procedure as described for confirming the histidine requirement. The non R-factor strain, TA 1535 was tested on the same plate as a control for ampicillin activity.

Determination of antimutagenicity against direct acting mutagens

Plate incorporation method was done for antimutagenicity assay without microsomal activation. Fresh bacterial cultures of *S. typhimurium* strains TA 1535 and TA 98 (1-2x109cells/ml) were mixed with 2ml of molten agar containing 0.5 mm histidine/biotin solution, different concentration of flower extract (0.1-1 mg/plate) and direct acting mutagens such as sodium azide (2.5µg/plate), Ethidium bromide (2.5µg/plate) or Hydroxyl amine (1µg/plate). Further it was spread over minimal glucose agar plates. Plates were incubated for 48 hours at 37ºC and the revertant colonies were counted.

RESULTS AND DISCUSSION

Genotype testing

Histidine requirement

Growth was observed only on Histidine/Biotin plates for tester strains TA 98, TA100 & TA1535 after 24 hours incubation at 37^{0} C. Control plates did not show growth indicating the absolute requirement of histidine/biotin for the strains to grow.

rfa Mutation

The tester strains TA 98, TA 100 & TA 1535 showed a clear zone of inhibition appeared around the crystal violet disc after incubation at 37^0 C for 24 hours. The clear zone indicated the presence of rfa mutation, which permitted large molecules such as crystal violet to enter and kill the bacteria. This confirmed rfa mutation.

TABLE I : Antimutagenicity of *Caralluma adscendens Roxb* extract against NaN_3, Ethidium bromide & Hydroxyl amine on *Salmonella typhimurium* strain TA 1535.

GROUP	average no. of colonies			% inhibition		
	Sodium azide	Ethidium bromide	Hydroxyl amine	Sodium azide	Ethidium bromide	Hydroxyl amine
Control	205	250	290			
100 µg	96	103	95	53	59	67
250 µg	55	73	78	73	70	73
500 µg	40	46	47	80	81	84
1 mg	12	7	12	94	96	96

TABLE II : Antimutagenicity of *Caralluma adscendens roxb* extract against NaN_3 Ethidium bromide & Hydroxyl amine on *Salmonella typhimurium* strain TA 100.

GROUP	average no. of colonies			% inhibition		
	Sodium azide	Ethidium bromide	Hydroxyl amine	Sodium azide	Ethidium bromide	Hydroxyl amine
Control	260	212	270			
100 µg	120	93	108	54	56	60
250 µg	58	61	81	78	71	70
500 µg	34	26	53	87	88	80
1 mg	7	3	17	97	98	93

UVrB Mutation

TA 98, TA 100 & TA 1535 showed growth only on unradiated side of the plates. The irradiated side of the plates did not show any growth. This indicates the UV sensitivity of the organism due to UVrB deletion.

R- Factor

Growth was observed in the ampicillin plates along the streaks made with TA 98 and TA 100. No growth was observed for TA 1535. TA 98 and TA 100 are R-factor strain. This strain possessed PKM 101, DNA essential for ampicillin resistance. No growth was seen on TA 1535 as it had no R-factor and it was tested as a control for ampicillin sensitivity. The R-factor served as a convenient marker that made it possible to test for the presences of plasmid.

TABLE III: Antimutagenicity of *Caralluma adscendens roxb* plant extract against NaN_3, Ethidium bromide & Hydroxyl amine on *Salmonella typhimurium* strain TA 98.

GROUP	average no. of colonies			% inhibition		
	Sodium azide	Ethidium bromide	Hydroxyl amine	Sodium azide	Ethidium bromide	Hydroxyl amine
Control	95	198	121			
100 µg	71	102	76	25	48	37
250 µg	48	81	47	49	59	61
500 µg	27	30	21	71	85	82
1 mg	6	16	14	94	92	88

Results of antimutagenic studies (given in table I, II & III) revealed that ethanolic extract of Caralluma adscendens Roxb. was highly effective in reducing the mutagenicity caused by the mutagens namely sodium azide , ethidium bromide and hydroxyl amine.

The percent inhibition of sodium azide induced mutagenicity was recorded as 94 % in TA 1535, 97 % in TA100, 94 % in TA98. The percent inhibition of ethidium bromide induced mutagenicity was recorded as 96 % in TA 1535, 98 % in TA102, 92 % in TA98. The percent inhibition of hydroxyl amine induced mutagenicity was recorded as 96 % in TA 100, 93 % in TA102, 88 % in TA98.

All the strains demonstrated reduction in the revertant colonies in a dose-dependent manner. So antimutagenic effect of the caralluma extract is based on the type of mutagen used and dose levels.

The antimutagenic factors are divided into two main classes according to differences in their modes of action: one type of factor is the desmutagen, which inhibits the formation of mutagens out of the cell or taking the mutagens into the cell, or inactivates or destroys mutagens directly or indirectly out of the cell, and the other type of factor is called a bio- antimutagen, which suppresses the process of mutagenesis itself in the cell, for example, it eliminates radicals or increases DNA repair systems. Recent research has confirmed that plant flavonoids inhibit the mutagencity induced by chemical mutagens [10].

The pregnane glycosides of *Caralluma* have been shown to possess antitumor and anti-cancer activities and in some studies *Caralluma* is reported to protect gastric mucosa and have antiulcer properties [11].

Natural substances such as flavonoids and tannins or their derivatives, present in these samples, were previously described as possessing antimutagenic properties and these metabolites could be involved in mutagen deactivation [12].

Anticarcinogenic and antimutagenic activity of medicinal and food plants may be due to a variety of mechanisms such as inhibition of genotoxic effects, inhibition of cell proliferation, signal transduction modulation, scavenging of free radicals, induction of detoxification enzymes, induction of cell-cycle arrest and apoptosis, modulation of cytoskeletal proteins that play a key role in mitosis, and the inhibition of topoisomerase I or II activity [13].

Hence, the possible mechanism of the demonstrated antimutagenic behaviour could be due to the bioactive constituents like flavone glycosides, pregnane glycosides, saponins, tannins, bitter principles, megastimane glycosides and sitosterols present in the ethanol fraction which might inactivae the reactive intermediates formed from mutagens.

CONCLUSION

The results showed that most significant inhibition of mutagenicity induced to TA 100 by the direct acting mutagens such as sodium azide and ethidium bromide. It also showed significant inhibition to the mutagenicity induced to TA 1535 by direct acting mutagen hydroxyl amine. There results indicated possible antimutagenicity activity of the compounds present in *Caralluma adscendens Roxb.*

The above results indicate that the *Caralluma adscendens Roxb.* extract could inhibit the mutagenicity induced by direct acting mutagen in the *Salmonella typhimurium* strain.

The use of antimutagens and anticarcinogens in the diet has been suggested as the most effective procedure for cancer prevention. Chemoprevention and dietary modification studies are underway to identify promising candidates for reduced cancer risk. The current work on identification of antimutagens from *Caralluma adscendens Roxb.* may play a role in improving human health.

REFERENCES

[1] Bhatia. A, Arora.S, Nagpal.A, Singh.B, Ahuja. SP, *J. Chin. Clin. Med.*, **2007**, 2, 8,428-434.
[2] Azadmehr. A, Hajiaghaee.R, Afshari. A, Amirghofran. Z, Kopaei. R , Darani. HY and Shirzad. H, *J. Med. Plants Res*., **2011**, 5, 11, 2365-2368.
[3] Shona MY, Choib SD, Kahngb GG, Namb SH, Sunga NJ, *Food Chem. Toxicol*., **2004**, 42,659–666.
[4] Bala .S, Grove .I S , *Mutat. Res*.,**1989** 222,141-8.
[5]. Zahin .M, Aqil .F, Ahmad.I, *Mutat .Res*., **2010,** 703, 99–107.
[6],Negi .PS, Jayaprakash .GK, Jena BS, *Food chem*.., **2003** ,80,393.
[7] Ghazali1.R, Abdullah .R, Ramli. N, Rajab .NF, Ahmad-Kamal .MS, and Yahya .NA, *J. Med . Plants Res.*, **2011** 5, 8, 1345-1348.
[8].Reid .KA, Maes .J, Maes A, Staden. JV, Kimpe ND, Mulholland DA, Verschaeve. L , *J. Ethnopharmacol*., **2006**,106,44–50.
[9].Vajha.M, Amrutha.V, Audipudi, Murthy. KSR, *Asian J. of biochemical and Pharmaceutical Research*, **2011,** 1, 2,500-506.
[10]. Miyazawa. M & Hisama .M, *Biosci, Biotechnol, Biochem*., **2003**, 67, 10, 2091-2099.
[11].Mahmood.T, Muhammad.S, Shinwari.ZK., *pak. J. Bot*., **2010,** 42, 2, 1163-1171.
[12].Horn .RC and Vargas .VMF, *Mutagenesis*, **2002,** 18, 2,113-118.
[13] Zahin.M, Ahmad.I, Aqil. F.,*Toxicol. in Vitro*.,**2010** 24 ,1243–1249