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Antimutagenic Effect of Medicinal Plants from Alternative Medicine

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Research Article

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Abstract

Aqueous extracts of Chirayita (Swertia chirayita Linn.) whole herb, Banafshah (Viola odorata Linn.) leaves and flowers and Mameeran (Coptis teeta Roxb.) rhizome were tested for their anti-mutagenic potential by Ames Salmonella Histidine point mutation assay of Maron, et al. with partial modifications as described by Kaur, et al. At a dose of 50 µg/plate, crude drug extracts exhibited the inhibition of His+ revert ants from 36.59% to 96% against direct acting mutagen sodium azide (NaN3) which induced mutagenicity in Salmonella typhimurium tester strains TA97a, TA98 and TA100. However, at concentrations (5 and 25 µg/ plate) of the plant extracts, a significant decrease in anti-mutagenic activity was recorded. In the present findings, herbal drug extracts at tested concentrations showed no sign of mutagenicity to the tester strains. Potent anti-mutagenic activity of Banafshah was observed followed by Chirayita and Mameeran. Linear regression analysis of the data shows dose dependent anti-mutagenic activity of the extracts. Qualitative analysis reveals the presence of active phytochemical as amarogentin, mangiferin (Chirayita), Cyclotides (Banafshah), Berberine (Mameeran) along with other phyto-constituents flavonoids, phenols in the tested extracts which are responsible for their anti-mutagenic activity.

Keywords: Herbal Drugs; Anti-Mutagenic; *Salmonella Typhimurium*

Introduction

Mutagens are known to prompt gene mutations or alteration in cells and thus, may affect somatic and germinal cells. Any mutations at the level of germinal cells might be inherited by next generations. Unfortunately, the degree of exposure of mankind to mutagens is still high [1]. Since mutagens are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bio-active phyto-compounds in counteracting the pro mutagenic and carcinogenic effects is gaining credence. Physical or chemical agents that reduce the mutagenicity of physical and chemical mutagens are called as anti-mutagens, which have been first reported almost four decades ago. Since then numerous studies have been carried out in order to identify compounds which might protect humans against DNA damage and its consequences [2].

Currently there is renewed interest in the study and use of medicinal plants because such investigations provide new leads on novel, active molecules of therapeutic importance. Anti-mutagenic and anti-carcinogenic properties of

wide variety of dietary constituents and plant secondary metabolites have been reported [3,4]. Han, et al. [5] reported that dietary polyphenols can modulate diverse biochemical processes involved in carcinogenesis. Various synthetic drugs and analogs are present to combat such diseases, but widespread and often indiscriminate use of such drugs together with poor hygiene, many mutagens have acquired resistance to specific treatments and these resistant strains are particularly evident in hospital environment. So, in view of increase in side effects due to the use of Western Medicines and increase in the resistance pattern to these drugs in mutagen developing organisms there is a need to search for the drugs of natural origin to combat such diseases.

The long history of the use of plants in traditional medicine systems makes this a valuable area for study and the importance of development of a broad range of drugs used in modern medicine is well known [6]. Plants from traditional medicines of India have been reported which have great potential for combating cancer include *Podophyllum hexandrum, Colchicum luteum, Nothaprdytes foetida, Bauhinia purpurea, Celestrus paniculatus, Argemme mexicana* [7]. And, last four decades have particularly witnessed a vast search of plant kingdom for substances that have anticancer activity and clinical studies have been conducted on a number of promising compounds isolated from *Catharanthus roseus, Podophyllum* spp., *Taxus baccata, Camptotheca acuminata, Cephalotaxus harringtonia* var. *drupacea* [8]. To date, natural products from medicinal plants still remain a solid niche as a source from which cancer therapies can be derived [9].

In Unani medicine there are reports of many herbs having potential anticancer agents [10] and there is a need to put them on scientific testing and validate the claims. Therefore, based on combination of literature reports on plant extracts pharmacology, phytochemistry and ethno medical claims, three prospecting Unani medicinal plants, namely, Banafshah (*Viola odorata* L.), Chirayita (*Swertia chirayita* L.) and Mameeran (*Coptis teeta* Roxb.) were investigated. The objective of the present study was to analyze the potential of these herbs for their efficacy to kill or inhibit the growth of cells systematically for anti-mutagenic activity using Ames *Salmonella* test assay against directly acting mutagens. The results are presented in this communication.

Alternative Medicine and Classical Unani Literature to Combat Cancer

Alternative Medicine that includes the Traditional System of Medicine (TSM) comprises of Ayurveda, Unani, Siddha and Homeopathy (AYUSH) in India utilizes drugs of natural origin. Unani System of Medicine (USM) is traditional medicine of Greeco-Arab origin in which more than 80% of drugs are of plant origin along with mineral and animal origin drugs. Vast classical literature is available in USM authored by eminent Unani physicians including Discords (90 A.D.), Hippocrates (460-377 B.C.), Avicenna (980-1037 A.D.), Galen (131-210 A.D.), Rhazes (850-925 A.D.) Hakim Ajmal Khan (1868-1927) and others who have mentioned the use of Unani drugs in various diseases including cancer and cancer like conditions, although these diseases were not known as such by present day modern medical terms but as '*Amraaz e Saudavia'* in USM. Various Unani text as De Materia medica (*Kitabul Hashaish)* by Discords, Canon of Medicine (Al-Qanoon) by Ibne Sina, Galen Pharmacopeia etc. gives valuable information on medicinal plants and their use in such diseases. The classical literature justifies that drugs in USM have been used since ancient times which is not solely based on empiricism, and this is evident from the fact that many medicinal plants, which were used in Greeco-Arab period still find place in modern therapy.

Material and Methods

Collection of Plant Material

The study was conducted at the department of Ilmul Advia (Unani Pharmacology and Pharmaceutical Sciences), Aligarh Muslim University, Aligarh (U.P) INDIA during the years 2009-2010.

The crude drug material of three test drugs under study was procured from different sources in India. Chirayita was procured from the local market of Baradari in Aligarh City (Uttar Pradesh); Mameeran from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh City (Uttar Pradesh) and Banafshah from the local market of Srinagar (Jammu & Kashmir).

Test drugs were authenticated by Pharmacognosy section in the department of Ilmul Advia. Voucher specimens of Banafshah (*Viola odorata* Linn.)/Family-Violaceae/SC-0099/09-V; Chirayita (*Swertia chirayita* Linn.)/Family-Gentianaceae/SC-0100/09-G; and Mameeran (*Coptis teeta* Roxb.)/Family-Ranunculaceae/SC0101/09-R was deposited in museum of the department of Ilmul Advia, Aligarh Muslim University and Aligarh for future reference and is represented in (Figure 1).

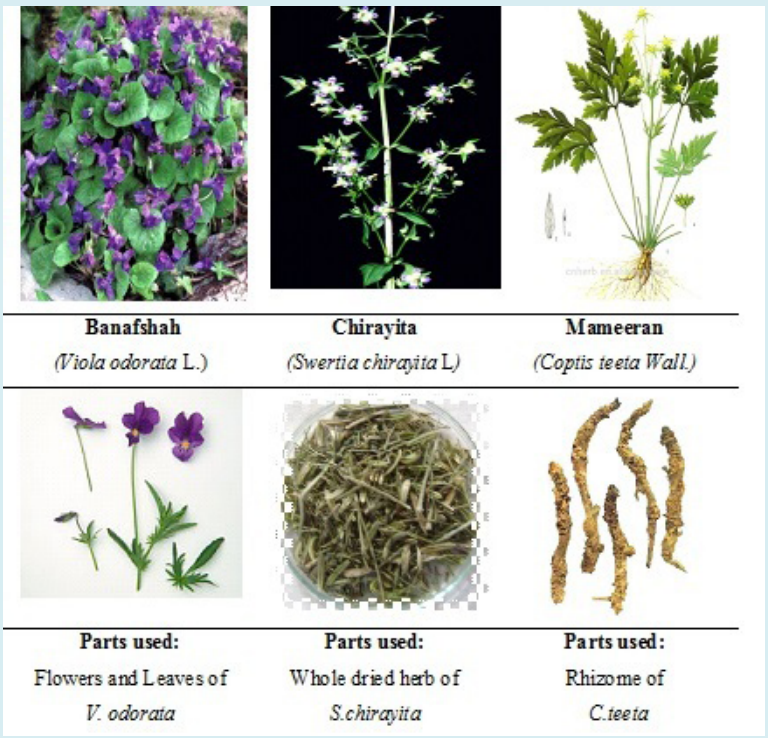

Figure 1: Test drugs of Unani medicine.

Preparation of Plant Extracts

The plant extracts were prepared as described by Jenkins. et al. [11] with slight modification of keeping temperature to a maximum of 45^0 C to avoid the loss of thermo labile elements. Dried plant powder (100 gm) was extracted using soxhlet apparatus by reflux method with double distilled water (250 ml) as a solvent at 45^0C for 6 hours or until the extracting return in the siphon was colorless. Aqueous extract so obtained was concentrated to dryness under vacuum in Lyophilizer (Macro Scientific works, New Delhi) under reduced pressure and reconstituted in 1 ml of Dimethylsulphoxide (DMSO) and stored at -20°C for further use [12,13].

Bacterial Strains and Chemicals

Authenticated *Salmonella typhimurium* strains TA97, TA98 and TA100 were obtained from department of Agricultural Microbiology, Aligarh Muslim University and Aligarh, INDIA. The bacterial strains were maintained in frozen stocks and grown as described by Maron, et al. [14].

Sodium Aside was procured from Himedia labs Pvt. Ltd., Mumbai, INDIA. All solvents and other chemicals used were of analytical grade and obtained from Merck, Sigma and Hi-media Labs.

Anti-mutagenicity Assay

Salmonella Histidine point mutation assay of Maron, et al. [14] was used to test the anti-mutagenic activity of the extracts, with some modifications as described by Kaur, et al. [15]. In the ant mutagenicity test, the inhibition of mutagenic activity of the sodium aside by the test samples was determined.

In the pre-incubation experiment, a mixture of test plant extract and mutagen, each having a volume of 0.1 ml in varying concentrations viz. 5, 25 and 50 µg from a stock solution of 10 mg/ml was pre-incubated at 37^0C for 30 min before addition to the bacterial culture, followed by addition of 2.5 ml of top agar containing 0.5% of NaCl and 0.6% agar supplemented with 0.5mM Histidine-biotin. The combined solutions were vortexes and poured onto minimal glucose agar plates (having 40% glucose solution and 50X Vogel Bonner medium). The plates were incubated at 37°C for 48 hours, after which the numbers of Histamine-independent revertants colonies were scored. Bacterial survival was routinely monitored for each experiment. To check the toxicity of the test samples, parallel controls were also run with the extracts alone at all concentration tested with mutagens. The concentration of the test sample for investigating the ant mutagenicity was 5, 25 and 50 µg/plate. Sodium was used as a diagnostic mutagen (1.0 µg/plate) in

positive control and plates without test samples and DMSO only were considered as negative controls in TA 97a, TA98 and TA100 tester strains. All the test samples and mutagens were dissolved in DMSO. Each sample was assayed using duplicate plates and the data represented here are mean of three independent assays. Inhibitory activity is expressed as percentage decrease of reverse mutation.

Percentage Inhibition (% inhibition)

The calculation of % inhibition was done according to the formula given by Ong, et al. % inhibition = [1-T/M] / 100 T= Number of revertants per plate in presence of mutagen and test sample. M = Number of revertants per plate in positive control.

The number of spontaneous revertants was subtracted from numerator and denominator. The anti-mutagenic effect was considered moderate when the inhibitory effect was 25-40% and strong when more than 40%. An inhibitory effect of less than 25% was considered as weak and was not recognized as positive [16].

Anti-Mutagenic Index

The number of his+ revert in treated plates was compared to negative control by its anti-mutagenic index value.

$$\text{Anti-Mutagenic index} = \frac{\text{Number of his + revertants induced in the treated plates}}{\text{Number of his + revert induced in the negative control}}$$

Statistical Analysis

The results are presented as the average and standard error of three experiments with triplicate plates/dose/ experiment to determine the significance of the number of his revert in sample as compared to control, one way ANOVA was done at P≤0.05 using software.

Results

Aqueous extracts of 'Chirayita' (*Swertia chirayita* Linn.)-leaves, 'Banafshah' (*Viola odorata* Linn.)-leaves and flowers and 'Mameeran' (*Coptis teeta* Roxb.)- Rhizome was found to be non-mutagenic to *S. typhimurium* tester strains TA97a, TA98 and TA100 when tested by pre-incubation method. 'Banafshah' extract produced a potent antimutagenic activity with 98% inhibition of TA97a strain and 96% for TA98, while 77 % demonstrated for TA100 at 50 µg/plate. 'Chirayita' produced 98% inhibition against TA100, while 85% against TA98 and 68% to TA97a tester strains and 'Mameeran' extract causes 93% inhibition in TA100 strain, 90% in TA98 and 72% in TA97a tester strain Jurado, et al. [17].

Highest anti-mutagenic activity was observed in 'Banafshah' that might be due to the presence of exceptional chemical and biological stability of cyclotides which makes them interesting, in particular for their potential as pharmacological tools and possibly leads to antitumor agents whereas 'Chirayita' and 'Mameeran' also showed good anti-mutagenic activity. Cytokines and other phyto-constituents of chirayita i.e. amarogentin and mangiferin may be responsible for this activity in it. Berberine and proto berberine, the important class of alkaloids in 'Mameeran' can be responsible for its effective anti-mutagenic activity. Thus, all three plants extracts exhibited non-toxicity in *Salmonella* tester strains at all the tested concentrations by Ames test against sodium azide (NaN_3) induced mutagenicity (Figure 2). Present work is the first attempt to analyze ant mutagenic activity of three Unani drugs viz. *V.odorata*, *S.chirayita* and *C.teeta*; the significant ant mutagenic activity found in an increasing order is Banafshah > Chirayita > Ameren.

(Figure 2) Inhibition percentage of Test Drugs against *S. typhimurium* tester strains at various concentrations.

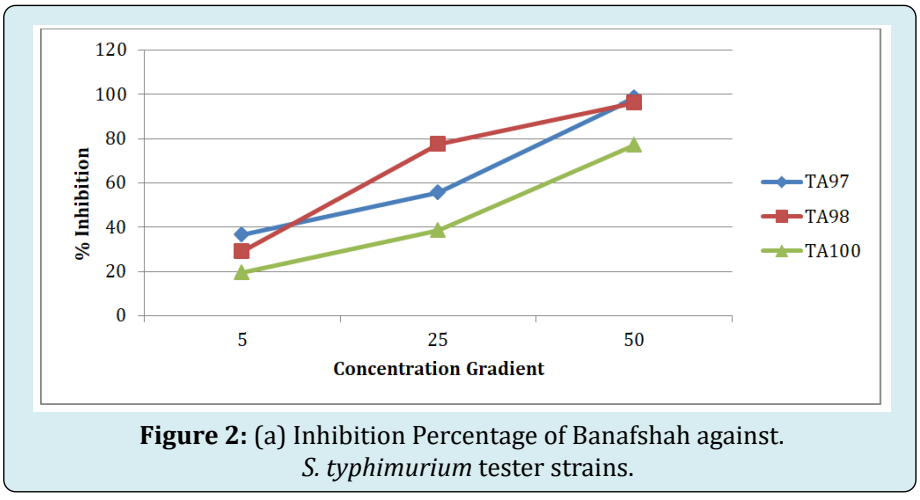

Figure 2: (a) Inhibition Percentage of Banafshah against. *S. typhimurium* tester strains.

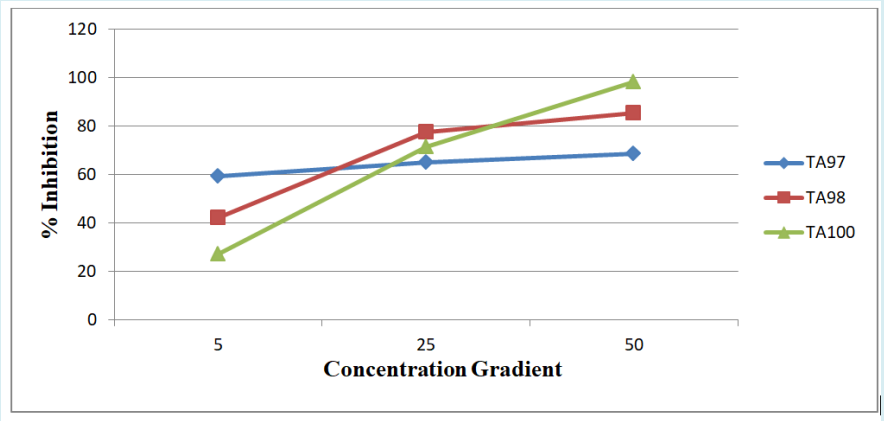

Figure 2: (b) Inhibition Percentage of Chirayita against. S. *typhimurium* tester strains

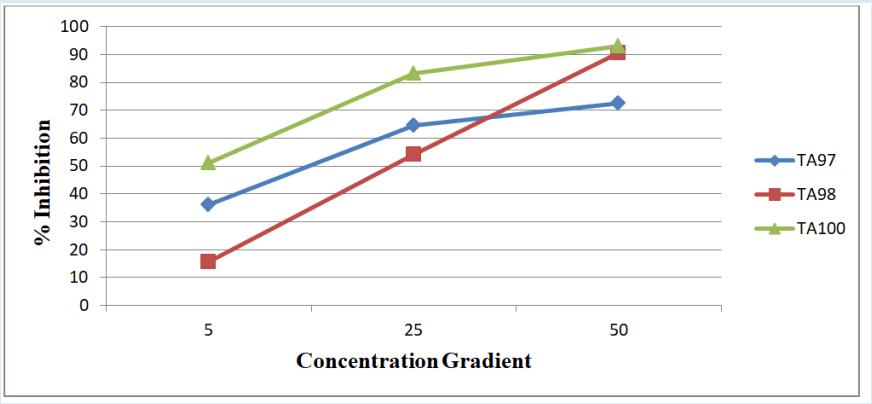

Figure 2: (c) Inhibition Percentage of Mameeran against. S. *typhimurium* tester strains

Discussion

The protective effect of different test drugs against the mutagenicity of Sodium azide was evaluated by Ames test using *S. typhimurium* TA97a, TA98 and TA100 (Table 1). It shows that potent anti-mutagenic activity of Banafshah displayed 98% inhibition of TA97a and 96% for TA98 while 77 % for TA100 at 50 µg/plate. These findings are in agreement with those of Perwaiz, et al. [18] who analyzed banafshah for its a*nti-tumorigenic effect of crude extract on DMBA-induced two stage skin carcinogenesis in Swiss albino mice and found that* cycloviolacin; a cyclotide from *V. odorata* is having antitumor effects and causes cell death by membrane permeabilization. The study also reveals several cycloids with robust cytotoxicity that may be promising chemo-sensitizing agents against drug resistant breast cancer. Lindholm, et al. [19] investigated cytotoxic activities of three naturally occurring macro cyclic peptides (cycloids) isolated from the two violets, *Viola arvensis* Murr and *Viola odorata* L. using non fluoro metric micro culture assay in a panel of 10 human tumor cell lines and found that all the three cyclotides, varv A, varv F, and cycloviolacin O2 exhibited strong cytotoxic activities, which varied in a dose-dependent manner. Gerlach, et al. [20] also isolated cycloviolacin from *V. odorata* which has anti-cancerous and chemo sensitizing ability and suggested that several cycloids with robust cytotoxicity may have promising chemo sensitizing agents against drug resistant breast cancer. The exceptional chemical and biological stability of cycloids makes them interesting in particular for their potential as pharmacological tools and possibly as antitumor agents.

Drugs	Conc. (µgm/ plate)	Strains					
		TA 97a		TA98		TA100	
		AMi	% Inhibition	AMi	% Inhibition	AMi	% Inhibition
Banafshah (V.odorata)	5	0.14	36.59	0.22	28.94	0.22	19.58
	25	0.42	55.71	1.28	77.62	0.61	38.33
	50	0.63	98.44	1.96	96.04	0.84	77.06
Chirayita (S.chirayita)	5	0.31	59.27	0.14	42.1	0.24	27.06
	25	0.35	64.94	1.42	77.63	1.26	71.66
	50	0.4	68.55	1.77	85.52	1.41	98.33
Mameeran (C.teeta)	5	0.27	36.07	1.1	15.78	1.51	51.25
	25	0.35	64.43	6.415	54.21	2.29	83.33
	50	0.63	72.67	10.06	90.65	3.33	93.16

Table 1: Effect of aqueous plant extracts on the directly induced mutagen in *Salmonella typhimurium* tester strains.

Similarly Chirayita (*Swertia chirayita* Linn.) also exhibited 98% inhibition against TA100 strain, while 85% against TA98 and 68% in TA97a tester strain, this might be due to the presence of important phyto-constituents i.e. amarogentin and mangiferin which are responsible for the modulation of pro-inflammatory and immune-regulatory cytokines by its activity in modulating IL-1β, IL-4, IL-6, IL-10. TNF-α and IF-γ [21,22]. The study carried out by Kumar, et al. [23] also supports present findings of its anti-mutagenic activity to some extent and its usage in the prevention of arthritis in the broader aspect through its activity on associated cytokines. Ant carcinogenic activity of *S. chirayita* has also been evaluated by Saha, et al. [24] on DMBA-induced mouse skin carcinogenesis model that also supports our results.

Mameeran (*Coptis teeta* Wall.) have various phyto-active compounds, among them bebeerine has attracted our attention. Berberine is a major representative of protoberberine alkaloids, which exhibited diverse biochemical and pharmacological actions [25]. In the present study it produces 93% inhibition in TA100, 90% in TA98 and 72% in TA97a tester strains. Anti-mutagenic compounds act at cellular level by enhancing the activities of enzymes involved in detoxification of mutagens, inhibiting the activities of enzymes involved in formation of mutagens metabolites, trapping of electrophiles, scavenging reactive oxygen species, inhibiting metabolic activation, or protecting nucleophile sites of DNA [26]. Present findings are suggestive of the fact that the anti-mutagenic effect is due to the presence of various phyto-active compounds. Polyphenols, particularly flavonoids have an ideal structure for scavenging free radicals and also interact with the active groups of mutagens or protect the sites of DNA that would be affected by the mutagen [27,28]. The study confirms that plant origin drugs are a proven source of useful anti-mutagenic agents.

Conclusion

The significant anti-mutagenic activity has been observed in three Unani drugs, namely Banfshah (*Viola odorata* Linn.*)*, Chirayita (*Swertia chirayita* Linn.*)* and Mameeran (*Coptis teeta* Roxb.*)* in an increasing order respectively in the study, provide a need for further investigations of these drugs against both direct and S9 dependent mutagens in-vitro and in-vivo systems.

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