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Antimutagenicity of Extracts of Three Edible Plants from Mosul City (Iraq) in Conidia of *Aspergillus amstelodami*

Sahi J. Dhahi Abdulkareem F. Omar
Department of Biology
College of Science
Mosule University

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ABSTRACT

The potential antimutagenic action of extracts of three common edible plants in Mosul was tested in conidia of the ascomycetous fungus *Aspergillus amstelodami*. Extracts of leaves of celery(*Apium graveolens* Linn) and parsley (*Petroselinum sativum* Hoffn.) and the round roots of radish (*Raphanus sativus* Linn.) were made in water, methanol, ethylacetate or petroleum ether. The antimutagenic action was sought in the reduction of frequency of spontaneous and induced (by 8-Methoxypsoralen + Near UV, 8-MOP + NUV) mutants resistant to 8-azaguanine. Three final concentrations in the growth medium of each extract were tested. These were (µg/ml) 800, 1600 and 3200 for celery and parsley and 600, 800 and 1200 for radish. The water extract of celery was ineffective while its concentration 3200 in methanol in ethylacetate and all three concentrations in the petroleum ether were effective in reducing the frequency of induced resistant mutants. Extracts of parsley behaved similarly. Extracts of the radish roots were all ineffective at all concentrations tested except the concentration 1200 of the water extract. The effect of the radish water is attributed to peroxidases and oxidases while those of celery and parsley to some lipid-soluble components which were sequentially concentrated in the petroleum ether according to the extraction protocol used.

Furthermore, none of the extracts by itself or in combinations with 8-MOP alone or NUV alone was found effective in significantly altering the frequency of the spontaneous mutants. This was taken to indicate that the extracts by themselves did not act as mutagenis or synergistic mutagens or photosensitizers to NUV. Moreover, their action on the positive mutagen (8-MOP + NUV) and not on the spontaneous frequencies suggests that these extracts behaved as desmutagens acting on the mutagen rather than as bioantimutagens acting directly on DNA.

التأثير المضاد للطفرة الذي تبديه مستخلصات من ثلاث من نباتات الأكل في مدينة الموصل (العراق) في كونيدات الفطر *Aspergillus amstelodami*

الملخص

لقد جرى اختبار القدرة المضادة للتطفير لمستخلصات من ثلاث نباتات شائعة الاكل في مدينة الموصل (العراق) في كونيدات الفطر *Aspergillus amstelodami*. اذ جرى اختبار مستخلصات

الاوراق الخـضر الطريـة لكـل مـن نبـاتي الكـرفس (*Apium graveolens* Linn.) والمعـدنوس (*Petroselinum sativum* Hoffn.) والجـذور المـدورة لنبـات الفجـل (*Raphanus sativus* Linn.) المحضرة في اربع مذيبات هي الماء والمذيبات العضوية الكحول المثيلي وخلات الاثيل وايثـر البتـرول وذلك من خلال الخفض الحاصل في تكرار الطافرات التلقائية والمستحثة بواسـطة المعاملـة المطفـرة 8-Methoxypsoralen + Near UV (8-MOP + NUV) بين كونيدات الفطر المعاملـة بالمستخلـصات اعلاه وعند التراكيز النهائية (مايكروغرام/مل) 800، 1600 و 3200 لكل من الكـرفس والمعـدنوس و 600، 800 و 1200 لنبات الفجل. لم تظهر مستخلصات الفجل تاثيراً يذكر ماعدا عنـد اعلـى تركيـز 1200 من المستخلص المائي وذلك على التكرارات المستحثة فقط. وقد عزي ذلك الى وجـود انزيمـات Peroxidases والـ Oxidases التي تستخلص بالماء والتي ربما تكون قد مسخت بالمـذيبات العـضوية ففقدت تاثيرها في هذه المستخلصات. اما المستخلصات المائية لنباتي الكرفس والمعدنوس فلم تبدِ تـاثيراً مضاداً للطفرة ولكن ظهر مثل هذا التاثير عند اعلى تركيز (3200) من مستخلص الميثـانول وخـلات الاثيل بينما اظهرت جميع التراكيز المختبرة من مستخلص ايثر البترول تـاثيراً واضـحاً وايـضاً ضـد الطفرات المستحثة فقط دون التلقائية. وقد عزي ذلك الى وجود مواد ذائبة في الدهون والمذيبات العضوية المستخدمة حيث يزداد تركيزها من الميثانول الى ايثر البترول حسب بروتوكول الاستخلاص المتسلـسل المستخدم.

لم يؤدِ أي من المستخلصات سواءاً بمفرده او بالاشتراك مع 8-MOP لوحده او مع NUV لوحدها الى زيادة في تكرار الطافرات التلقائية مما يشير الى عدم سلوك المستخلـصات كمطفـرات بمفردهـا او بالتآزر مع 8-MOP وعدم سلوكها كمحسسات ضوئية لـ NUV . اضـاف الـى ذلـك فـان تـاثير المستخلصات على تكرار الطافرات المستحثة دون التلقائية يشير الى ان هذه المستخلـصات قـد سـلكت سلوك مضادات تطفير ظاهرية (Desmutagens) تؤثر على المطفر وليس كمـضادات تطفيـر حقيقيـة (Bioantimutagenis) تعمل على DNA الطافر نفسه او الذي في مرحلة الطفور.

INTRODUCTION

Our genetic material is daily exposed to a variety of of genotoxic agents with the consequent accumulation of mutations that can lead to a variety of hereditary diseases, teratogenesis or carcinogenesis (Brusick, 1980; Moutschen, 1985). Living organisms, however, are provided with a whole range of defensive mechanisms against these genotoxic agents; some are intrinsic (e. g. various repair mechanisms) and others are extrinsic (e. g. chemopreventers) (De Flora and Ramel, 1988).

Chemopreventers (CP) are chemicals that act as antimutagens/anticarcinogens and widely distributed in our natural foods especially diets of plant origin (e. g. fruits, vegetables, cereals, tea, coffee… etc.) (Bronzetti, 1994; Bronzetti et al., 1992; Stavric, 1994). Therefore, there has been a surge in the search for identifying these chemicals in various edible plants using crude extracts as a first step and proceeding to isolate them in

a pure state in the second stage (Mitscher et al., 1986; Weisburger, 2001; Lohman et al., 2001).

The aim of the present work is to test the antimutagenic activity of three vegetable plants widely used in daily food in Mosul City (North of Iraq); celery (*Apium graveolens*, Linn) represented by its fresh green leaves, parsley (*Petroselinum sativum*, Hoffn) represented also by its fresh green leaves, and garden radish (*Raphanus sativus*, Linn) represented by its round roots. In each case, crude extract of the plant materials in water, methanol, ethylacetate or petroleum ether was used to treat mutagenized and unmutagenized conidia of the fungus *Aspergillus amstelodami*. The antimutagenic effect of each extract was sought in the reduction in frequency of mutants resistant to 8-azaguanine among the extract-treated as compared with the untreated conidia.

MATERIALS AND METHODS

1- Test organism : Tests for antimutagenicity were done in conidia of the strain A1(*wA*) of the ascomycetous fungus *Aspergillus amstelodami.* This is a prototrophic strain having the mutation *wA* that renders the conidial colour white in contrast to the olive green colour of the wild type (Caten, 1979). It is also sensitive to the base analogue 8-azaguanine being inhibited by as low a concentration as 10 μg/ml (Dhahi and Caten, 1987).

2- Media and microbiological techniques : culturing conditions and microbiological techniques used are, basically, those of Caten (1979). Tests were done on the minimal (M) medium or its derivative MD (M containing the salt sodium deoxycholate, D, at a final concentration of 400 μg/ml). The MD was used when a large number of small discrete colonies per plate were needed. Incubations were done at 30^{o}C, the optimal growth temperature of the fungus.

3- Stock solutions : A stock solution of the toxic analogue 8-azaguanine containing 500 μg/ml in distilled water was prepared according to Hoffman and Malling (1974). A stock solution of the photosensitizer 8-methoxypsoralen (8-MOP) (Fluka, Switzerland) containing 200 μg/ml in absolute ethanol was prepared according to Alderson and Scott (1970).

4- Plant materials : Samples of fresh green leaves of celery (*Apium graveolens*, Linn) and of parsley (*Petroselinum sativum*, Hoffn); and of fresh round roots of radish (*Raphanus sativus*, Linn) were collected from local markets of the Mosul city, Northern Iraq. The identities of the three plant species were confirmed at the Herbarium, Department of Biology, College of Science, University of Mosul. Leaves and roots were washed with tap water and dried. The materials were put in nylon bags and kept in the refrigerator until extraction.

5- Preparation of plant extracts : Extraction was made in distilled water or in organic solvents. Three organic solvents were employed; absolute methanol, ethylacetate and petroleum ether. Water extracts were made according to the method of Riose et al.,

(1988). Extracts were lyophilized and kept as powders in tightly closed glass containers until use.

Organic-solvent extracts were made according to the methods of Botting et al., (1999). Extraction was carried out sequentially with increasing hydrophobicity starting with methanol, then, ethylacetate and lastly with petroleum ether. Plant extract from each solvent was lyophilized and kept freez-dried in tightly closed glass container until use. All in all 12 extracts (3 plants x 4 extracts) were prepared

6- Preparation and sterilization of plant extract stock solutions : A stock solution of each of the 12 lyophilized extracts was prepared at a final concentration of 200 mg/ml. The solvents were distilled water for the water extracts, 50% methanol for the methanol extracts and dimethylsulfoxide (DMSO) for the ethylacetate and petroleum ether extracts. All solutions were sterilized by pasteurization at 62^{o}C for 10 minutes (Botting et al., 1999). Moreover, all growth media were supplemented with 0.05 mg/ml chloramphenicol to reduce bacterial contamination.

7- The minimal inhibitory concentrations (MICs) of the extracts : These were done for each extract by point inoculation of the tester strain A1 on M and M containing ascending concentrations of the extract and measuring the inhibition (% reduction in colony diameter on the extract medium as compared with that on M). Three sublethal concentrations (causing no more than 10% reduction in diameter) from each extract were chosen for subsequent antimutagenicity tests. These were (µg/ml); 800, 1600 and 3200 for *A. graveoleus* and *P. sativum*; and 600, 800 and 1200 for *R. sativus*.

8- The positive mutagenic treatment : In addition to their effect on the frequency of spontaneous mutants, the extracts were tested for their potential antimutagenic effect on the frequency of mutants induced by a known mutagenic treatment. The positive mutagenic treatment consisted of 15 min exposure of conidial suspensions at 20 cm distance to long wave UV or far UV (FUV) from a CH-335 Galenkamp UV lamp emitting most of it light at a wavelength of 365 nm. Before irradiation, the conidia were photosensitized with the coumarin 8-methoxypsoralen (8-MOP) by preincubating the suspension for 15 min at room temperature at a final concentration of 8-MOP of 100 µg/ml. The treatment 8-MOP + NUV was found to be highly mutagenic in conidia of *Aspergillus nidulans* (Alderson and Scott, 1970) and in conidia of *Aspergillus amstelodami* (Dhahi, 1978). The genetic endpoint sought in the present work was the recovery of mutants resistant to the analogue 8-azaguanine (*azg*). This is a very suitable system for estimating the frequency of forward point mutations in *A. nidulans* (Morpurgo, 1962).

9- The antimutagenicity tests : Basically, these were done as described by Mitscher et al., (1986). A conidial suspension (~ 60 ml) containing about 10^7 conidia/ml (haemocytometer count) was prepared from 3-day old colonies of strain A1. The suspension was partitioned into four 5-ml samples and four 10-ml samples. The first of the 5-ml samples was used without any treatment and constituted the spontaneous or negative control. The second, third and fourth samples were preincubated for 20 min wth

800, 1600 and 3200 µg/ml of extract of *A. graveolens* or *P. sativum* respectively; or with 600, 800 or 1200 µg/ml of *R. sativus* extract respectively.

The first of the 10-ml suspension samples was treated with 8-MOP + NUV (as above) and constituted the positive mutagenic treatment. The other 3 samples were preincubated with the plant extracts as in the first group above before they were subjected to the 8-MOP + NUV treatment. These three samples were used to detect any antimutagenic effect that might be exerted by the extracts on the positive mutagenic (8-MOP + NUV) treatment.

The eight conidial suspensions described above represented the undiluted (10^0) samples and each one was used to prepare serial dilutions in distilled water down to 10^{-4}. Five MD + Azg (25 µg/ml) were each inoculated with 0.1 ml aliquot to the 10^0 suspension and 5 MD plates were similarly inoculated but from the 10^{-4} dilution from each of the 8 treatments. The 10^{-4} plates were used for viability count that was used to estimate the population size of viable conidia plated on the 10^0 plates. Colonies growing on the azaguanine medium were counted and represented the resistant mutants. Their total on the five plates was divided by the expected population on the five 10^0 plates to give the frequency of resistant mutants from each treatment. This protocol was repeated three times for each extract and the mean frequency of mutants was estimated. The means of the three extract treatments were each statistically, using *t* test, compared with the spontaneous treatment. The means of the three extract with 8-MOP + NUV were each similarly compared with the positive control (8-MOP + NUV) treatment. This protocol was repeated for all four different extracts of each of the three edible plants.

RESULTS AND DISCUSSION

Results of testing the antimutagenicity of water extracts of the three plants are shown in Table 1. In each plant, treatment 1 represents the spontaneous (negative control) while treatment 5 (8-MOP + NUV) represents the positive mutagenic treatment. The mutagenic effect of the latter treatment is indicated by the highly significant difference, as shown by the *t* test, between its mutant frequency and that of the spontaneous treatment. This was evident in all three sets of comparison (three extract concentrations) (Table 1).

Preincubating conidia with just the water extract at the final concentration (µg/ml) of 800, 1600 or 3200 of *A. graveolens* (AG) or *P. sativum* (PS) or 600, 800 or 1200 of *R. sativus* (RS) did not cause significant difference in the mutant mean frequencies between these treatments and their respective spontaneous controls (treatments 2, 3 and 4 for each extract; Table 1). When preincubation with water extract was followed by exposure to 8-MOP + NUV (treatments 6, 7 and 8 in each extract) the outcome varied, however. Water extracts of AG or PS and at all concentrations used caused no significant reduction in the frequencies of the 8-MOP + NUV treatments but that of RS at its highest concentration (1200 µg/ml) did cause a significant reduction in the frequency of the mutants (12.4 ± 0.15) as compared to the positive control (15 ± 0.61) ($t_{(4)} = 10.00$; $P < 0.01$) (Table 1).

Effect on mutant frequencies by methanol, ethylacetate or petroleum ether extracts was variable. Extracts of *R. sativus* did not affect the spontaneous nor the induced frequencies (Tables 2,3,4). Extracts of *A. graveoleus* and *P. sativum* did not affect the spontaneous frequencies. Methanol and ethylacete extracts of the two plants, however, reduced the induced frequencies but only at the highest concentration (3200 µg/ml) used

(Tables 2, 3 and 4). The petroleum ether extract significantly reduced the induced mutant frequencies and at all three concentrations tested. The reduction increased with rising concentrations exhibiting a dose-response relationship (Tables 2, 3 and 4).

Table 1 : Effect of the water extract of the plants *Apium graveolens* (AG), *Petroselinum sativum* (PS) or *Raphanus sativus* (RS) on the frequency of azaguanine-resistant (*azg*^{r}) mutants in the unmutagenized and 8-MOP + NUV mutagenized conidia of *Aspergillus amstelodami*.

Plant Extract	**Treatment (concentration of extract (µg/ml))**	**Mutant frequency (x 10^{-6})**			**(Mean ± SE) (x 10^{-6})**	**$t_{(2+2)}$[(a)]**
		R1	**R2**	**R3**		
AG	*1. O*	1.5	1.6	1.4	1.5 ± 0.06	
	2. 800	1.7	1.8	1.6	1.7 ± 0.06	2.45
	3. 1600	1.7	1.9	1.5	1.7 ± 0.12	1.68
	4. 3200	1.6	1.7	1.8	1.7 ± 0.06	2.45
	5. 8-MOP + NUV	16.3	17.6	17.4	17.1 ± 0.40	38.62**
	6. 8-MOP+ NUV + 800	17.3	16.5	17.2	17.0 ± 0.25	0.21
	7. 8-MOP+NUV+1600	17.4	15.6	1.8	16.9 ± 0.68	0.51
	8. 8-MOP+NUV+3200	17.6	16.1	16.4	16.7 ± 0.46	1.27
PS	*1. O*	1.7	1.6	1.6	1.6 ± 0.03	
	2. 800	1.5	1.5	1.2	1.4 ± 0.14	1.43
	3. 1600	1.6	1.4	1.5	1.5 ± 0.06	1.56
	4. 3200	2.1	1.7	1.9	1.7 ± 0.12	0.83
	5. 8-MOP + NUV	17.8	17.4	17.6	17.6 ± 0.12	132.83**
	6. 8-MOP+ NUV + 800	18.2	16.6	17.0	17.3 ± 0.48	0.60
	7.8-MOP+NUV + 1600	17.5	17.4	16.8	17.2 ± 0.22	1.61
	8.8-MOP+NUV + 3200	17.3	17.3	17.0	17.2 ± 0.10	2.62
RS	*1. O*	1.1	1.8	1.5	1.5 ± 0.20	
	2. 600	1.8	1.9	1.5	1.7 ± 0.13	1.07
	3. 800	1.5	1.9	1.5	1.6 ± 0.14	0.41
	4. 1200	1.5	1.96	2.2	1.9 ± 0.18	1.46
	5. 8-MOP + NUV	14.8	15.3	15.6	15.2 ± 0.23	44.07**
	6. 8-MOP+ NUV + 600	16.1	14.0	14.9	15.0 ± 0.61	0.31
	7. 8-MOP+NUV + 800	15.3	14.2	15.1	14.9 ± 0.34	0.73
	8. 8-MOP+NUV+1200	12.3	12.7	12.2	12.4 ± 0.15	10.00**

(a) $t_{(2+2)}$ is the statistics for 4 degrees of freedom. SE, Standard Error

For each extract, treatments (2), (3), (4) and (5) are statistically compared with treatment (1) (the spontaneous frequency) while treatments (6), (7) and (8) are compared with treatment (5) (the induced frequency).

** significant at 1%.

R1, R2 and R3 are replicates.

Table 2 : Effect of the methanol extract of the plants *Apium graveolens* (AG), *Petroselinum sativum* (PS) or *Raphanus sativus* (RS) on the frequency of azaguanine-resistant (*azg*^{r}) mutants in the unmutagenized and 8-MOP + NUV mutagenized conidia of *Aspergillus amstelodami*.

Plant Extract	**Treatment (concentration of extract (µg/ml))**	**Mutant frequency (x 10^{-6})**			**(Mean ± SE) (x 10^{-6})**	**$t_{(2+2)}$ [(a)]**
		R1	**R2**	**R3**		
AG	*1. O*	1.5	1.5	1.7	1.6 ± 0.07	
	2. 800	1.5	1.1	1.5	1.4 ± 0.14	1.31
	3. 1600	2.0	1.6	1.6	1.7 ± 0.14	0.66
	4. 3200	2.2	1.3	1.6	1.7 ± 0.14	0.66
	5. 8-MOP + NUV	15.2	14.9	14.2	14.8 ± 0.29	43.21**
	6. 8-MOP+ NUV+800	14.6	14.8	14.4	14.6 ± 0.14	0.61
	7.8-MOP+NUV+1600	13.4	13.3	13.8	13.5 ± 0.15	3.89*
	8.8-MOP+NUV+3200	10.8	11.1	11.4	11.1 ± 0.17	10.76**
PS	*1. O*	1.6	1.5	1.8	1.6 ± 0.09	
	2. 800	1.6	1.2	1.6	1.5 ± 0.14	0.61
	3. 1600	1.7	1.6	1.9	1.7 ± 0.09	0.78
	4. 3200	1.5	1.8	1.9	1.7 ± 0.12	0.66
	5. 8-MOP + NUV	14.5	13.9	14.4	14.3 ± 0.19	61.00**
	6.8-MOP+ NUV+ 800	14.1	14.7	13.8	14.2 ± 0.27	0.31
	7.8-MOP+NUV+1600	13.6	17.0	14.4	14.0 ± 0.23	1.01
	8.8-MOP+NUV+3200	12.0	12.4	11.9	12.1 ± 0.15	9.11**
RS	*1. O*	1.6	1.8	1.8	1.7 ± 0.07	
	2. 600	1.1	1.2	2.2	1.5 ± 0.35	0.56
	3. 800	1.4	2.2	1.7	1.8 ± 0.24	0.65
	4. 1200	1.3	1.7	1.8	1.6 ± 0.08	0.93
	5. 8-MOP + NUV	11.7	13.1	13.5	12.8 ± 0.55	20.15**
	6. 8-MOP+NUV+ 600	12.4	13.2	13.4	13.0 ± 0.31	0.32
	7. 8-MOP+NUV+ 800	13.4	11.9	12.9	12.7 ± 0.44	0.14
	8.8-MOP+NUV+1200	12.8	13.6	11.7	12.7 ± 0.55	0.13

Symbols are as in table 1.

Table 3 : Effect of the Ethyl acetate extract of the plants *Apium graveolens* (AG), *Petroselinum sativum* (PS) or *Raphanus sativus* (RS) on the frequency of azaguanine-resistant (*azg*^{r}) mutants in the unmutagenized and 8-MOP + NUV mutagenized conidia of *Aspergillus amstelodami*.

Plant Extract	**Treatment (concentration of extract (µg/ml))**	**Mutant frequency (x 10^{-6})**			**(Mean ± SE) (x 10^{-6})**	$t_{(2+2)}$ **(a)**
		R1	**R2**	**R3**		
AG	*1. O*	0.4	2.3	1.9	1.5 ± 0.58	
	2. 800	0.4	1.6	1.7	1.2 ± 0.41	0.47
	3. 1600	0.4	1.9	1.6	1.3 ± 0.46	0.27
	4. 3200	2.2	1.7	1.3	1.7 ± 0.26	0.63
	5. 8-MOP + NUV	15.4	14.7	13.9	14.7 ± 0.43	18.31**
	6. 8-MOP+NUV+ 800	15.6	14.3	15.3	15.1 ± 0.39	0.68
	7.8-MOP+NUV+1600	15.3	14.2	13.9	14.5 ± 0.72	0.24
	8.8-MOP+NUV+3200	4.8	6.9	7.4	6.4 ± 0.79	9.15**
PS	*1. O*	1.8	1.5	1.9	1.7 ± 0.12	
	2. 800	1.1	1.9	1.5	1.5 ± 0.23	0.76
	3. 1600	1.7	1.6	0.9	1.4 ± 0.25	1.07
	4. 3200	2.5	1.4	1.0	1.6 ± 0.45	0.21
	5. 8-MOP + NUV	15.5	15.6	15.7	15.6 ± 0.06	102.65**
	6. 8-MOP+NUV+ 800	15.4	15.8	16.4	15.9 ± 0.29	1.00
	7.8-MOP+NUV+ 600	15.4	16.0	15.2	15.5 ± 0.24	0.40
	8.8-MOP+NUV+3200	10.7	10.8	10.9	10.8 ± 0.06	58.82**
RS	*1. O*	1.5	1.9	1.7	1.7± 0.12	
	2. 600	1.9	1.5	2.1	1.8 ± 0.18	0.47
	3. 800	1.6	2.0	1.7	1.8 ± 0.12	0.59
	4. 1200	1.6	1.8	1.3	1.6 ± 0.15	0.53
	5. 8-MOP + NUV	16.3	14.2	13.5	14.7 ± 0.84	15.30**
	6. 8-MOP+NUV+ 600	14.2	14.0	15.5	14.6 ± 0.47	0.10
	7. 8-MOP+NUV+ 800	14.1	14.6	14.2	14.3 ± 0.15	0.47
	8.8-MOP+NUV+1200	13.3	14.0	14.7	14.0 ± 0.40	0.75

Symbols are as in table 1.

Table 4 : Effect of the petroleum ether extract of the plants *Apium graveolens* (AG), *Petroselinum sativum* (PS) or *Raphanus sativus* (RS) on the frequency of azaguanine-resistant (*azg*^{r}) mutants in the unmutagenized and 8-MOP + NUV mutagenized conidia of *Aspergillus amstelodami*.

Plant Extract	**Treatment (concentration of extract (µg/ml))**	**Mutant frequency (x 10^{-6})**			**(Mean ± SE) (x 10^{-6})**	**$t_{(2+2)}$ [(a)]**
		R1	**R2**	**R3**		
AG	*1. O*	1.8	1.5	1.9	1.7 ± 0.13	
	2. 800	1.4	1.8	2.2	1.8 ± 0.23	0.38
	3. 1600	1.4	1.4	1.8	1.5 ± 0.14	1.07
	4. 3200	1.1	1.9	1.8	1.6 ± 0.25	0.35
	5. 8-MOP + NUV	17.3	17.3	15.4	16.7 ± 0.63	23.19**
	6. 8-MOP+NUV+ 800	13.1	12.3	11.2	12.2 ± 0.59	5.19**
	7.8-MOP+NUV+1600	9.7	8.7	9.1	9.2 ± 0.29	10.75**
	8.8-MOP+NUV+3200	8.3	8.9	8.8	8.7 ± 0.19	12.11**
PS	*1. O*	1.5	1.1	1.9	1.5 ± 0.23	
	2. 800	1.5	1.5	0.8	1.3 ± 0.24	0.60
	3. 1600	1.2	1.5	1.2	1.3 ± 0.10	0.79
	4. 3200	1.2	1.6	1.95	1.6 ± 0.22	0.32
	5. 8-MOP + NUV	18.4	16.9	17.1	17.5 ± 0.47	30.51**
	6. 8-MOP+NUV+ 800	15.9	13.9	12.1	13.7 ± 0.87	14.99**
	7.8-MOP+NUV+1600	11.1	11.3	9.3	10.6 ± 0.64	13.45**
	8.8-MOP+NUV+3200	9.5	7.9	7.7	8.4 ± 0.57	11.22**
RS	*1. O*	1.2	1.5	1.6	1.4 ± 0.12	
	2. 600	1.6	1.6	1.5	1.6 ± 0.04	1.55
	3. 800	1.3	1.6	1.5	1.5 ± 0.09	0.81
	4. 1200	2.0	1.2	1.6	1.6 ± 0.23	0.77
	5. 8-MOP + NUV	15.3	13.7	16.6	15.2 ± 0.84	16.28**
	6. 8-MOP+ NUV+600	15.6	14.9	15.7	15.4 ± 0.25	0.23
	7. 8-MOP+NUV+ 800	15.5	14.7	15.1	15.1 ± 0.23	0.12
	8.8-MOP+NUV+1200	14.5	14.9	14.4	14.6 ± 0.15	0.70

Symbols are as in table 1.

Both *A. graveolens* and *P. sativum* contain considerable amounts of vitamin C soluble in water (Hussein, 1985). This vitamin is recognized for its antimutagenic/anticarcinogenic activity through inactivating free radicals formed during food oxidation (Hodges, 1982). Failure of water extracts of these two plants to reduce spontaneous or induced mutant frequencies (Table 1) could be attributed to the oxidation of vitamin C by exposure to atmospheric oxygen during the process of extraction (Hayastu et al, 1988). The water extract of *R. sativus* roots is rich in both vitamin C and the enzymes peroxidases and oxidases (Hussein, 1985) but as the vitamin C is oxidized

during extraction as indicated above, the antimutagenic action of *R. sativus* water extracts at the highest concentration (1200 µg/ml) on the induced of mutants (Table 1) could be attributed to the peroxidases and oxidases. It has been reported that these two types of enzymes are strong antioxidants and hence can strongly inactivate mutagens/carcinogens (Kada and Shimoi, 1987; DeFlora and Ramel, 1988).

Leaves of *A. graveolens* and *P. sativum* are rich in many lipid-soluble (organic-solvent extractable) components with clear antimutagenic/ anticarcinogenic activities. These include chlorophylls and flavonoids acting as antioxidants or by trapping free radicals (Stavric, 1994; Botting et al., 1999; Huang et al., 2004), β-carotene, vitamin A, and other carotenoid derivatives also acting as antioxidants or free-radicals trappers (DeFlora et al., 2001; Ferguson, 2001; Jang and Surh, 2001). Other lipid-soluble components of *A. graveolens* and *P. sativum* extracts that have been reported to be antimutagenic include lycopene, lipids with unsaturated fatly acids, terpenes and d-limonene (Josephy et al., 1990 and Stavric, 1994). The antimutagenic effect against the induced mutants exhibited by extracts of these two plants in methanol (Table 2), ethylacetate (Table 3) and petroleum ether (Table 4) could be attributed to one or another of the lipid-soluble components above. As extraction was done sequentially from the least extracting solvent (methanol) to the petroleum ether the strongest among the three solvents in extracting the most hydrophobic (lipid-soluble) substances, components of the two plants would be mostly concentrated in petroleum ether. This could explain why the antimutagenicity of the methanol and ethylacetate extracts was evident only at their highest (3200 µg/ml) concentrations (Tables 2 and 3) while all concentrations tested from the petroleum ether extracts were effective (Table 4). Roots of *R. sativus*, on the other hand, are devoid of these lipid soluble components (Hussein, 1985). Moreover, vitamin C would be oxidized during extraction and peroxidases and oxidases in the roots, being proteins, might have been denatured by the three organic solvents and lost activity. Therefore organic-solvent extracts of *R. sativus* showed no antimutagenic activities against induced mutants (Tables 2, 3 and 4).

None of the extracts had significantly increased the spontaneous frequencies of the mutants (Tables 1, 2, 3, and 4) and hence none of them was mutagenic by itself. None of the extracts had significantly reduced the spontaneous frequencies either. All extracts, by one test or another, however did show significant reductions in the induced mutants (Tables 1, 2, 3 and 4). This demonstrates that the extracts contained component (s) that have acted as desmutagens (interacting with the mutagens) rather than as true bioantimutagens (working on the mutating or mutated DNA itself) (Kada & Shimoi, 1987; Mitscher et al, 1986; Weisburger, 2001). The suggested mechanism of 8-MOP + NUV mutagenesis is that 8-MOP has two functional groups that can be activated by NUV to bind to a thymine on one strand to form a monodduct, and on further exposure to NUV a second thymine on the opposite strand will bind to 8-MOP to form a biadduct or interstrand cross links (Ben-Hur and Song, 1978). Both monoadducts and cross links are mutagenic in various organisms (Islas et al, 1991; Yandell et al, 1994). The active component (s) in the present extracts might have interacted with the functional groups of the 8-MOP and prevented them from interacting with the thymine bases of DNA on exposure to NUV.

Moreover, psoralens themselves might undergo photochemical modifications or degradations by NUV leading to the formation of active products such as singlet oxygen

or free radicals that can induce damages and subsequent mutation in the DNA (Knox et al, 1988). Components of the plant extracts might have interfered with the production these active products or trap them.

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