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ANTIMICROBIAL AND ANTIMUTAGENIC ACTIVITY OF SOME MEDICINAL PLANTS

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ABSTRACT

The widespread use of medicinal plants for medical purposes has increased dramatically due to their great importance on the public health. In this study, three medicinal plants, *Anasatatic hierochuntica, Peganum harmala* L. and *Rosmarinus officinalis* L. were selected to evaluate their antimicrobial, mutagenic and antimutagenic activities.

The antimicrobial activity has been evaluated against some gram negative (*Pseudomonas aeruginosa, E.coli*), gram positive (*Staphylococcus aurues*) bacteria and bacteriophage F116. The results of inhibition zone and survival percentages indicated that, these plant extracts have a strong antimicrobial activity against the tested microorganisms. The killing effect was reached up to 98.14% against bacteria and 92.7% against bacteriophage F116. Mutagenic activity was evaluated using prophage induction and transduction assays. Neither released phage from lysogenic nor transductants cells have been observed in some treatments. This indicated that plant extracts have no mutagenic activity. The three plant extracts used in this study showed antimutagenic activity against the powerful mutagen agent, EMS.

Keywords: Antimicrobial, antimutagenic, medicinal plants, prophage induction, transduction.

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INTRODUCTION

Nature has been a source of medicinal agents since immemorial. Herbal medicine is still the mainstay of about 65-80% of the whole population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with human body and fewer side effects (Amal *et al.*, 2010). Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruits and bark can all be constituents of herbal medicines. The medical values of these plants lie in their phytochemical components,which produce definite physiological actions on the human body. The most important of these components are alkaloids, tannins, flavonoid, phenolic compounds (Sheriff, 2001). Phytochemicals are extensively found at different levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, toothache and rheumatism diseases (Exarchou *et al.*, 2002).

The antimicrobial properties of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Parekh *et al.*, 2005) However, *Anastatica hierochuntica, Peganum harmala* L. and *Rosmarinus officinalis* L. are widely used as medicinal plants either by themselves or in combination with other herbs. The whole plant of *A. hierochuntica* is commonly known as kaff maryam or rose of jerisho, which is a winter annual plant of the Shara-Arabian deserts and was prescribed in the Egyptian folk medicine as a charm for child birth (Rizk and El-Ghazaly, 1995). The aerial part of the kaff maryam is still attractive for Egyptian people as a remedy for asthma and some diseases of the respiratory system (Amal *et al.*, 2010).

Peganum harmala L. was first found in dray area of central Asia and southern USA (Lamchouri *et al.*, 2000 and Sobhani *et al.*, 2000).The plant has a wide spectrum of pharmacological actions, e.g., monoamine oxidase inhibition (Adell *et al.*, 1996), binding to benzodiazepine receptors (Baum *et al.*, 1996), antioxidative action (Tse *et al.*, 1991 and Hayet *et al.*, 2010), DNA topoisomerase inhibition in cancerous cell-lines (Yamada *et al.*, 2006), reduce the toxicity induced by thiourea (Hamden *et al.*, 2008) and good

antiviral and antibacterial activity (Hayet *et al.*, 2010).

Rosemary (*Rosmarinus officinalis* L.) originally comes from southern Europe (Hethelyi *et al.*, 1987). Its leaves and oil are commonly used as spice and flavoring agents in food processing due to its desirable flavor, antimicrobial and antioxidant activities this can be attributed to its phenolic diterpenes contents, such as rosmarinic acid and carnosol (Collins and Charles, 1987; Baratte *et al.*, 1998; Mangena and Muyima, 1999; Larran *et al.*, 2001). Moreover the leaves are used in the preparation of alcoholic beverages, herbal soft drinks, and cooked foods and sauces (Maistro *et al.*, 2010). In medicine, it is used as a stimulant of blood circulation, the heart and the nervous system, probably because of its camphor content. It is applied topically to treat articulation, muscular, rheumatic, and traumatic pains. It is also employed in lotions against baldness (Al-Sereiti *et al.*, 1999). In this study, the antimicrobial, mutagenic and antimutagenic activities of *Anastatica hierochuntica, Peganum harmala, and Rosmarinus officinalis* L. are evaluated using some microbial systems.

MATERIALS AND METHODS

This study was performed in Microbial Genetic Lab., Genetics Dept., Fac. Agric., Zagazig Univ., Egypt.

Growth Media

Nutrient agar (NA) and nutrient broth (NB) media were used. Soft agar (0.8% W/V agar) was prepared in distilled water and kept at 45°C on water bath. Streptomycin (10mg/ml) and ampcillin (2mg/ml) were added as sterilized solutions by filtration through 0.2µm filter membrane to media after autoclaving.

Plant Materials

Plant materials:

A. hierochuntica, P. harmala L. *and R. officinalis* L. are purchased from the Egyptian local market.

Microbial Strains

Pseudomonas *aeruginosa* strains (PAO1, MAM2) and bacteriophage F116 were obtained from M. Day, Univ. of Wales, Cardiff, UK. *Escherichia coli* and *Staphylococcus aureus* from Microbial Genetic Lab stocks,

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Extraction of Plant Materials

A. hierchuntica (whole dried plants), *P. harmala* L. (seeds), and *R. officinalis* L. (dried leaves), were grinded into a fine powder. The samples were extracted by using a protocol published by Jung, *et al.*, (2008) with some modification. Briefly, 100 g of plant sample was extracted with 600 ml of 70% ethanol, twice at room temperature for two days and filtration using Whatman membrane filter with pore size 0.45 µm. The filtrates were concentrated to dryness by rotary evaporation at 60 °C. Each extract was solublized in 10% dimethyl sulfoxide (DMSO) (Hayet *et al.*, 2010). These solutions were sterilized by filtration through filter membrane (0.2 µm Whatman) and stored at 4 °C.

Antimicrobial Activities

Antibacterial activity of the tested plants were measured by two methods as follows, determination of inhibition zones and survival percentages of bacterial strains.

Inhibition zones: bacterial strains were cultured in broth cultures for overnight to yield approximately 1 x 10^9 cfu/ml. Three ml of soft molten agar inoculated with 0.1ml of overnight tested bacterial cultures, vortexed and poured over NA medium plates. After 30 min., different concentrations of plant extracts (0, 10, 20, 30, 40, 50%) were spoted on three sites of the plate. The plates were incubated at 30 °C for *P. aeruginosa* and 37 °C for *E. coli* and *S. aureus* for 24h.

Survival percentages: the previous concentrations of plant extracts were added to NB medium, inoculated with equal volume of overnight bacterial cultures and incubated for 24h. Serial dilution were prepared, counts were recorded on NA medium. The survival was estimated as viable cells (cfu/ml),

$$\text{cfu/ml} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

The killing percent was calculated (S% of control – S% of treatment).

Antiviral activity: bacteriophage F116 lysate was treated with the same previous concentrations of plant extracts for 24h (Hassan and Khattab, 2007). The plaques were counted and the pfu/ml was calculated.

Mutagenic Activity

Prophage induction assay was used to assess the mutagenic activity of tested plants. The NB flasks with different concentrations of plant extracts were prepared, inoculated by MAM2 lysogenic strain and incubated at 30°C for 24h. A few drops of chloroform were added and centrifugated at 5000 rpm for 30 min. and filtred through 0.2 µm filter membrane (Whatman). The supernatant was collected, the plaque forming units (pfu/ml) were calculated for each concentration.

Phage Titration

The double agar layer method (Sharma *et al.*, 2002 and Payan *et al.*, 2005) was used. Serial hundred dilutions of phage lysate were prepared, equal volumes (0.1ml) of phage lysate and host cells (grown overnight in liquid medium) were mixed with 3ml of soft molten agar. The mixture was vortexed and poured immediately onto NA medium plate. Plates were incubated at 30°C for overnight. Plaques were counted and the pfu/ml was calculated.

Transduction Assay

Recipient cells were grown in NB overnight, equal volumes (1ml) of phage lysate and recipient cell suspension were mixed. The mixture was kept for 15-30 min at room temperature to allow phage adsorption. Serial dilutions were prepared and placed onto selective medium (NA + streptomycin + ampcillin). Number of transductants were recorded and the transduction frequency was calculated (Hassan and Amin, 2010).

Positive and Negative Control

Ethylmethane sulfonate (EMS) was used as a positive control with prophage induction from lysogenic strain and transduction assay. One ml of EMS was added to 100 ml distilled water to prepare a stock solution 1%. Ascorbic acid (vitamin C) was used as a negative control. One gram of ascorbic acid was dissolved in 100 ml distilled water as a stock.

Antimutagenic Activity

Three concentrations, 20, 30 and 50% of *A. hierochantical, P. harmala* L. *and R. officinolis* L. extracts, respectively, were used against 10% of EMS. These concentrations were used because they appeared approximately LD_{50}. The lysogenic strain was treated with plant extracts before and post its treatment with EMS, the mixture of plant extracts and EMS were also used.

RESULTS AND DISCUSSION

Antibacterial Activity

The results of antibacterial activity are presented in Figure 1. Plant extracts of the three plants showed inhibition effects against antibiotic resistant strains of *P. aeruginosa*. Inhibition zone diameter caused by *A. hierochantical* and *P.harmala* L. extracts was reached to 3cm , but with *R . officinalis* L. was 2.5 cm. In contrast, inhibition zone diameter of the streptomycin with streptomycin sensitive strain was 3 cm and some sensitive colonies in the center have been detected. The use of ampcillin against ampicillin resistant strain did not show any inhibition. These results indicated that plant extracts from three plants can be used as antibacterial agents. According to Amal *et al.*, 2010, who reported that inhibition zone diameters was as follows: negative = 0 mm, weak = 1-4 mm, moderate = 5-10 mm, strong = 10-15mm, very strong = ≥16 mm.

The survival and killing percentages were also calculated. The effect of plant extracts of the tested plants on survival percentages of three different bacterial genera (*P. aeruginosa, E. coli and S. aurues*) was investigated. The results presented variable effects against tested bacteria. Survival percentages of *P. aeruginosa* was ranged from 1.86% to 51.06% when using *A. hierochantica*, from 3.45 to 34.8 % when using *P.harmala* L. and from 31.6 to 57.18 % when using *R. officinalis* L. (Table 1). The killing effect of 50% of plant extracts was very high, reached 98.14% upon using *A.hierochantica*, 96.55% upon using *P.harmala* L. and 68.4% upon using *R. officinalis* L. Survival percentages of *E. coli and S. aurues* was also decreased by increasing the concentration (Tables 2 , 3). These results gave another proof that the tested plants have a strong antibacterial agents.

The antibacterial activities against both gram negative (*P. aeruginosa* and *E. coli*) and gram positive bacteria (*S. aurues*) may indicated that the presence of broad spectra antibiotic components or simply metabolic toxins in plant extracts (Moniharapon and Hashinaga, 2004). It has been suggested that antibacterial activity of the *R . officinalis* L. extract may be ascribed mainly to the action of carnosic acid and carnosol [Oluwatuyi *et al.* (2004) and Bernardes *et al.* (2010)]. Hayet *et al.*

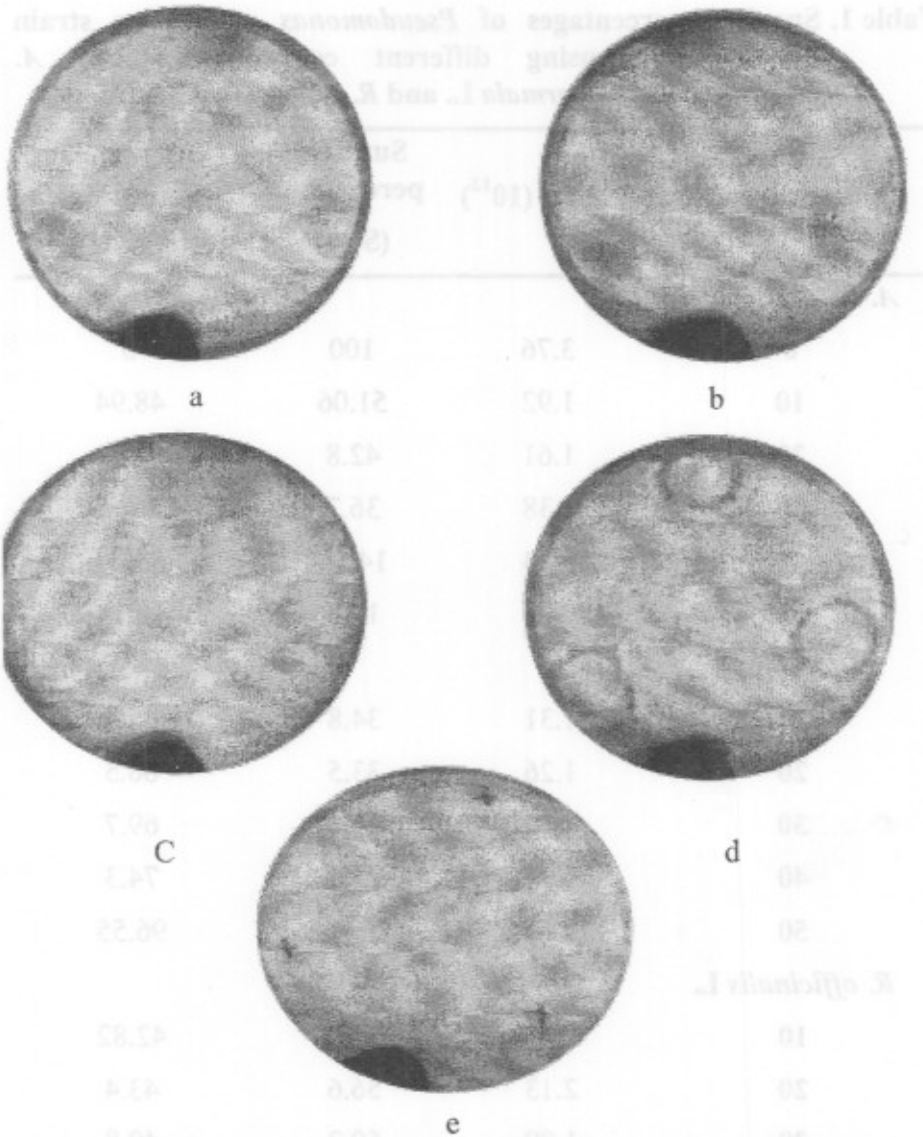

Fig. 1. Inhibition zone result from bacterial treatment with plant extracts: (a) effect of *A. hierochantica* on ampcillin resistant strain (b) effect of *P .harmala* L. on ampcillin resistant strain (c) effect of *R. officinalis* L. on ampcillin resistant strain (d) effect of ampcillin on ampcillin sensitive strain (e) effect of ampcillin on ampcillin resistant strain

Table 1. Survival percentages of *Pseudomonas aeruginosa* strain PAO1 upon using different concentrations of *A. hierochantica, P. harmala* L. and *R. officinalis* L. extracts

Concentration%	Cfu/ml (10^{12})	Survival percentage (S %)	Killing percentage (K%)
A. hierochantica			
0	3.76	100	0
10	1.92	51.06	48.94
20	1.61	42.8	57.2
30	1.38	36.7	63.3
40	0.54	14.36	85.64
50	0.07	1.86	98.14
***P. harmala* L.**			
10	1.31	34.8	65.2
20	1.26	33.5	66.5
30	1.14	30.3	69.7
40	0.97	25.7	74.3
50	0.13	3.45	96.55
***R. officinalis* L.**			
10	2.15	57.18	42.82
20	2.13	56.6	43.4
30	1.89	50.2	49.8
40	1.44	38.2	61.8
50	1.19	31.6	68.4

Table 2. Survival percentages of *E. coli* upon using different concentrations of *A. hierochantica, P. harmala* L. and *R. officinalis* L. extracts

Concentration%	Cfu/ml (10^{12})	Survival percentage (S%)	Killing percentage (K%)
A. hierochantica			
0	2.099	100	0
10	1.98	94.3	5.7
20	1.24	59.07	40.93
30	0.95	45.25	54.75
40	0.91	43.33	56.67
50	0.62	29.53	70.47
***P. harmala* L.**			
10	1.99	94.8	5.2
20	1.78	84.8	15.2
30	1.62	77.17	22.83
40	0.81	38.5	61.5
50	0.53	25.25	74.75
***R. officinalis* L.**			
10	1.99	94.8	5.2
20	1.90	90.5	9.5
30	1.89	90.04	9.96
40	1.48	70.5	29.5
50	1.11	52.8	47.2

Table 3. Survival percentages of *Staphylococcus aurues* upon using different concentrations of *A. hierochantica* , *P. harmala* L. and *R. officinalis* L. extracts

Concentration%	Cfu/ml (10^{12})	Survival percentage (S%)	Killing percentage (K%)
A.hierochantica			
0	2.98	100	0
10	1.96	65.7	34.3
20	1.31	43.9	56.1
30	0.98	32.88	67.12
40	0.15	5.03	94.9
50	0.12	4.02	95.9
***P. harmala* L.**			
10	1.69	56.7	43.3
20	1.58	53.02	46.9
30	0.99	33.22	66.78
40	0.98	32.88	67.12
50	0.63	21.14	78.8
***R. officinalis* L.**			
10	2.3	77.18	22.8
20	1.91	64.09	35.9
30	1.82	61.07	38.9
40	1.65	55.36	44.64
50	1.51	50.06	49.94

(2010), suggested that, antibacterial activity of aerial part of *P.harmala.* might be attributed to the high quantity of polyphenols which are known to possess efficient antibacterial activity.

Antimicrobial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls, cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nuclide acids (Oyaizu *et al.*, 2003).

The results obtained in study agreed with Oluwatuyi *et al.* (2004), who found antibacterial activities of *R. officinalis* L. extract against *S. aureus*. In addition, Faixova and Faix (2008) reported that rosemary essential oil had antimicrobial activity.However Bernardes *et al.*, (2010) found that extract from leaves of *R. officinalis* L. displayed higher antibacterial activity than the stems extract, against *streptococcus mutans, S.salivarius, S. sanguinis* and *Enterococcus faecalis.*

Hayet *et al.* (2010) found that methanol and chlorophorm extracts of the leaves of *P. harmala.* displayed higher antibacterial activity against gram positive (*S. aureus, Bacillus subtilus, Enterococcus faecalis*) than gram negative bacteria (*E. coli, Serratia marcescens, Klebsiella pneumoniae*). Amal *et al.* (2010) observed that, *A. hierochuntica* extract displayed antibacterial activity against gram positive bacteria (*B.subtilis*), not on gram negative bacteria (*E. coli, P. aeruginosa*) or on fungus (*A. niger*).

Antibiotic resistance was increased worldwide in both outpatients as well as hospitalized patients. In the last two decades, the problem escalated as the prevalence of antibiotic resistant bacteria has increased and multi-drug resistant strains have emerged in many species that cause diseases in human. There are no treatments available for infections caused by many of the antibiotic resistant bacteria, and resistance to commonly used antibiotics is steadily increasing. So, the extracts of three tested plants may be beneficial when used therapeutically in human. Data in Table 4 show the effect of EMS on survival of *P. aeruginosa* PAO1 strain.

Antiviral Activity

Pseudomonas aeruginosa phage F116 was used to study the antiviral activity of the tested plants. Bacteriophage lysate was treated with different concentrations (10–50%) of plant extracts. Results in Table 5 clarified that plant extracts affected on pfu/ml and subsequently on survival and killing

Table 4. Activity of ethylmethanesulfonate (EMS) on survival percentages of *Pseudomonas aeruginosa* strain PAO1

Concentration%	Cfu/ml	Survival percentage (S%)	Killing percentage (K%)
0	1.5×10^{10}	100	0.0
10	0.65×10^{10}	43.0	57.0
20	0.4×10^{10}	26.7	73.3
30	0.2×10^{10}	13.0	87.0
40	-	0.0	100
50	-	0.0	100

Table 5. Antiviral activity of *A. hierochantica* , *P. harmala* L. and *R. officinalis* L. plant extracts

Concentration %	Cfu/ml	Survival percentage (S%)	Killing percentage (K%)	No. of transductions (10^5)	Transduction frequency
A.hierochantica					
0	9.78×10^{11}	100	0.0	5.92	6.1×10^{-7}
10	6.99×10^{7}	71.5	28.5	0.387	0.402×10^{-8}
20	3.98×10^{7}	40.7	59.3	0.336	0.349×10^{-8}
30	3.12×10^{7}	31.9	68.1	0.298	0.309×10^{-8}
40	2.31×10^{7}	23.6	76.4	0.236	0.245×10^{8}
50	1.95×10^{7}	19.9	80.1	0.219	0.227×10^{-8}
***P. harmala* L.**					
10	3.51×10^{7}	35.9	64.1	0.469	0.487×10^{-8}
20	2.84×10^{7}	29.04	70.96	0.418	0.434×10^{-8}
30	1.26×10^{7}	12.9	87.1	0.278	0.288×10^{-8}
40	0.98×10^{7}	10.1	89.9	0.221	0.229×10^{-8}
50	0.71×10^{7}	7.3	92.7	0.24	0.249×10^{-8}
***R. officinalis* L.**					
10	7.99×10^{7}	81.7	18.3	0.598	0.621×10^{-8}
20	5.23×10^{7}	53.5	46.5	0.521	0.541×10^{-8}
30	4.82×10^{7}	49.3	50.7	0.428	0.444×10^{-8}
40	4.59×10^{7}	46.9	53.1	0.398	0.413×10^{-8}
50	2.86×10^{7}	29.2	70.8	0.311	0.323×10^{-8}

pfu/ml of recipient at zero time = 9.62×10^{12}

percentages. The killing percentage ranged from 28.5 to 80.1, 64.1 to 92.7 and 18.3 to 70.8% when using *A. hierochuntica*, *P. harmala* L. and *R. officinalis* L. respectively. These results show that the plant extracts have a remarkable antiviral activity. The antiviral activity is probably duc to the high phenolic content and the presence of polar substances such as flavonoids and tannins which are known to possess antiviral activity (Hayet *et al.*, 2010). Choi *et al.* (2008) found that *R. officinalis* L. extract possess antiviral activity against Enterovirus 71, and suggested that extract may contain compound inhibiting its replication. Hayet *et al.* (2010) found that methanol extract of *P. harmala* . displayed good antiviral activity against HCMV. Data in (Table 6) show the effect of EMS on phage F116.

Phage particles treated by plant extracts have been allowed to transduce streptomycin resistant gene. The number of transductants are affected and subsequently transduction frequency. This indicated that the transducing particles are affected.

Mutagenic Activity

Prophage induction assay

Data in Table 7 appeared mutagenic activity of three tested plant extracts using prophage induction assay. The concentrations ranged from 10–50% did not increase the induction of prophage from the lysogenic strain, in the mean time, prophage induction was decreased when the concentration was increased. However, the pfu/ml ranged from 2.97×10^9 to 0.11×10^9, from 1.37×10^9 to 0.0 and 1.8×10^9 to 0.54×10^9 when using *A. hierochuntica, P. harmala* L. and *R. officinalis* L., respectively. There was no fold increase in pfu/ml over the spontaneous release of phage from the lysogenic strain. So, the tested plant extracts do not possess mutagenic activity.

Data in Table 8 represent the activity of negative and positive control on prophage induction. No mutagenic effect of ascorbic acid (negative control) was appeared. EMS was recommended as a powerful effect as a mutagenic agent in the induction of the prophage. According to Rossman *et al.* (1985) and DeMarini *et al.* (1990), the positive response of any compound is recommended to be corresponding to a three-fold increase in pfu/ml over the spontaneous background release of the lysogenic strain.

Table 6. Antiviral activity of EMS

Concentration %	Cfu/ml	Survival percentage (S%)	Killing percentage (K%)	No. of transductions (10^5)	Transduction frequency
0	9.78×10^{12}	100	0.0	5.92×10^5	6.1×10^{-7}
10	7.86×10^7	80.4	19.6	1.3×10^3	1.35×10^{-10}
20	5.32×10^7	54.4	45.6	2.8×10^2	2.9×10^{-11}
30	0.49×10^7	5.01	94.99	-	-
40	-	0.0	100	-	-
50	-	0.0	100	-	-

pfu/ml of recipient at zero time = 9.62×10^{12}

Table 7. Prophage F116 induction from lysogenic strain by *A. hierochantica*, *P. harmala* L. and *R. officinalis* L. plant extracts

Concentration%	Cfu/ml (10^9)	Fold increase	Induced phage	Mutagenic response
A .hierochantica				
0	2.19	0	-	-
10	2.97	1.35	-	-
20	2.11	0.96	-	-
30	0.26	0.11	-	-
40	0.24	0.10	-	-
50	0.11	0.05	-	-
***P. harmala* L.**				
10	1.37	0.62	-	-
20	1.21	0.55	-	-
30	0.15	0.06	-	-
40	0.13	0.05	-	-
50	0.0	0.0	-	-
R. officinalis				
10	1.8	0.82	-	-
20	1.9	0.86	-	-
30	0.76	0.34	-	-
40	0.58	0.26	-	-
50	0.54	0.24	-	-

Table 8. Activity of ascorbic acid and elthylmethanesulfonate on prophage induction

Concentration%	Pfu/ml (10^9)	Fold increase or mutagenic index	Induced phage	Mutagenic response
0	1.63	0		
Ascorbic acid			-	-
10	1.69	1.03	-	-
20	1.54	0.94	-	-
30	1.22	0.74	-	-
40	1.19	0.73	-	-
50	1.13	0.69	-	-
EMS				
10	12.29	7.5	10.66	+
20	15.31	9.3	13.68	+
30	22.98	14.9	21.35	++
40	32.98	20.2	31.35	+++
50	31.9	19.5	30.27	+++

- < 3 = non mutagen
+ = 3 -10 = mutagen
+ + = 11 – 19 = high mutagen
+++ = > 19 = very high mutagen

Prophage induction and SOS response occur by a variety of mechanisms and involves a number of classes of genetic damage, including alkylation, cross-links, strand scissions and oxidative dominations (Elespuru, 1984 and Rossman *et al.* 1985). DNA strand breaks are inducer of prophage from lysogen, DNA topoisomeras II poisons and reactive oxygen species have been reported for their ability to induce the SOS response (Nakamura *et al.* 1987). In addition, the pattern of mutational damage induce is frequently similar to that induced by ionizing radiation and reactive oxygen species (Backer *et al.*, 1990, DeMarini and Lawrence, 1992). Accumulating evidences indicated that, prophage induction is a broader genetic endpoint than any other bacterial assay, since it is a sensitivate test, adding or no adding any substance to activate the result will not change the results. Sometime, the mutagenic evaluation of substances may escape when tested with Ames test, the mutagenic activity using *Salmonella* was negative, whereas

the prophage induction assay was positive (Varagas *et al.*, 2001; Cizmas *et al.*, 2003 and Kilgerman *et al.*, 2003).

Transduction assay

The effect of plant extracts on transducing streptomycin resistance gene was shown in Table 9. Number of transductants was decreased by increasing the concentrations and subsequently, resulting decreasing in transduction frequency. The frequency reached to 0.17×10^{-7} and 0.06×10^{-7} when using *A. hierochuntica* and *R. officinalis* L. extracts respectively. With *P. harmala* L. extract no transductants cells were detected upon exposure to high concentrations (40, 50%). No great enhancement was observed upon using ascorbic acid as a negative control Table 10. Transduction frequency has seriously increased from 2.42×10^{-7} to 8.7×10^{-7} by using EMS.

The low yield of transductants that appeared in this study, gave another proof that these plant extracts have no mutagenic activity especially when comparing these results with the results of the negative and positive control. The phage lysates that have been used in this experiment were collected from the lysogenic strain (MAM2) upon exposure to each treatment. Transduction frequency depends on transducing phage particles that may form among the whole phage particles produced from the lysogenic strain as it has been repeated by (Lang and Beatty, 2001; Miller 2001 and Kang *et al.*, 2002). For that, it seems that tested plant extracts did not increase the rate of host DNA fragmentation, number of transducing particles result in decrease in transduction frequency.

These results show that transduction analysis could be used as a bacterial tool in detecting the mutagenicity of compounds beside the known bacterial tools as it has been suggested by others (Mahmoud *et al.*, 2005).

Antimutagenic Activity

After antibacterial experiments were performed, the LD_{50} was determined per each plant extract and EMS. It was 20, 30, 50 and 10% of *A. hierochantica*, *P. harmala* L., *R. officinalis* L. and EMS respectively. Data in Table 11 appeared the results of antimutagenic activity using prophage induction and transduction assays. The lysogenic strain was

Table 9. Phage induction by plant extracts and transducing streptomycin resistance gene

Concentration	No. of transductions (10^3)	Transduction frequency (10^{-7})	Fold increase	Mutagenic response
A.hierochantica				
0	1.47	1.2	-	-
10	1.21	1.0	-	-
20	0.98	0.8	-	-
30	0.86	0.71	-	-
40	0.36	0.29	-	-
50	0.21	0.17	-	-
P. harmala L.				
10	1.1	0.9	-	-
20	0.53	0.43	-	-
30	0.16	0.13	-	-
40	0.0	0.0	-	-
50	0.0	0.0	-	-
R. officinalis L.				
10	1.56	1.28	-	-
20	0.91	0.75	-	-
30	1.0	0.82	-	-
40	0.19	0.15	-	-
50	0.08	0.06	-	-

Cfu/ml of recipient at zero time = 1.21×10^{10}

Table 10. Phage induction by ascorbic acid and EMS and transducing streptomycin resistance gene

Concentration %	No. of transductions (10^3)	Transduction frequency (10^{-7})	Fold increase	Mutagenic response
0	2.23	1.59	-	-
Ascorbic acid				
10	1.12	0.8	-	-
20	1.11	0.79	-	-
30	0.95	0.67	-	-
40	0.76	0.54	-	-
50	0.63	0.45	-	-
E M S				
10	3.4	2.42	1.5	-
20	5.8	4.1	2.57	-
30	9.9	7.07	4.44	+
40	12.3	8.7	5.47	+
50	10.7	7.6	4.7	+

Cfu/ml of recipient at zero time = 1.4×10^{10}

Table 11. Antimutagenic activity of *A. hierochantica* , *P. harmala* L. and *R. officinalis* L. plant extracts against EMS

Treatments	Pfu/ml	Fold increase	Mutagenic response	No. of transductants	Transduction frequency
Control	1.26×10^{12}	-	-	8.12×10^6	6.24×10^{-6}
EMS + *A. hierochantica*	1.59×10^7	-	-	0.0	0.0
EMS + *P. harmala* L.	1.26×10^9	-	-	0.0	0.0
EMS + *Rosmarinus officinalis* L.	1.22×10^9	-	-	0.0	0.0
Pre + *A. hierochantica*	2.51×10^5	-	-	3.9×10^2	3.0×10^{-10}
Pre + *P. harmala* L.	3.74×10^5	-	-	0.0	0.0
Pre + *Rosmarinus officinalis* L.	1.52×10^5	-	-	1.4×10^2	1.07×10^{-10}
Post + *A.hierochantica*	6.31×10^7	-	-	4.9×10^2	3.76×10^{-10}
Post + *P. harmala* L.	7.11×10^7	-	-	1.0×10^1	7.6×10^{-10}
Post + *Rosmarinus officinalis* L.	5.33×10^7	-	-	1.2×10^2	0.62×10^{-10}

Cfu/ml of recipient at zero time = 1.3×10^{12}

treated by plant extracts pre- and post-treatment by EMS, and so, mixture between them. Results of prophage induction indicated that, pre treatment was more effective than the other treatments, the Pfu/ml ranged from $1.52x10^5$ to $3.74x10^5$, whereas, in post treatment was $5.33x10^7$ to $7.11x10^7$. The mixture between plant extracts and EMS had a lower effect, the pfu/ml ranged from $1.59x10^7$ to $1.26x10^9$. In general, the pfu/ml was remarkable decreased compared with the effect of EMS only (Table 8). Number of transductants and transduction frequencies were also decreased. No transductant cells were observed in the mixed of EMS and plant extracts samples and so, in pre treatment with *P. harmala* L., it may due to the killing effect of EMS and plant extracts.

The results of prophage induction and transduction assayes in this experiment show that these plant extracts may have antimutagenic activity. So, *A. hierochantica, P. harmala* L. and *R. officinalis* L. can be used as a natural source.

The antimutagenic effects of plants can be attributed to wide range constituents such as chlorophyll, fibers and many phytochemicals including simple phenols, phenolic acids, flavonoids, tocopherols and ascorbic acid which known antioxidant (Mezzoug *et al.*, 2006). Chlorophyll and fibers act by scavenging of reactive molecules through binding or absorption. The fibers are able to absorb irreversibly the mutagens. Chloophylls and their soluble derivative chorophyllin inhibit genotoxicity by forming a reversible complex with the mutagenic agents. The phenolic compounds and many flavonoids were reported also to have the capacity to scavenge mutagens or free radicals (Rice – Evans *et al.*, 1996 and Yao *et al.*, 2004). The antimutagenic activity of some flavonoids was caused by radical scavenging effects (Edenharder and Grunhage, 2003). Other desmutagens were reported to be enzymatic modulators that can act through enzyme systems by inducing either phase 1 and /or phase II enzymes of detoxification or by altering the balance of different enzyme activities. These agents include ascorbic acid found in rosmary which reduce the mutagenic effect of MMS on *Drosophila* (Mezzoug *et al.*, 2006). Fahim *et al.* (1999) found that antimutagenic activity of rosemary ethanolic extract and essential oil are attributed to the presence of a relatively high

percentage of phenolic compounds with high antioxidant activity.

In contrast, Maistro *et al.* (2010) found that, *R. officinalis* essential oil induced significant increases in DNA damage (micronucli, chromosome aberrations) in mouse cells.

Hamden *et al.* (2008) found that ethanol and chlorophorm extracts of *P. harmala.* protected the animal cells against the carcinogenic effects induced by thiourea.

In conclusion, thc finding of study support the view that some medicinal plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. It can be also efficient as preventive agents that may be mutagens.

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النشاط المضاد للميكروبات والمضاد للطفور لبعض النباتات الطبية

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الاستخدام الواسع للنباتات الطبيبة فى الأغراض الطبية يزداد بشكل دراماتيكى بسبب أهميتها الكبيرة فى التأثير على الصحة العامة.

فى هذه الدراسة تم إختيار ثلاث نباتات طبية هى: كف مريم، الحرمل، روزمارى لتقييم الأنشطة المضادة للميكروبات والمضادة للطفور.

تم تقييم النشاط المضاد للميكروبات ضد بعض البكتريا السالبة لجرام (*Pseudomonas aeruginosa, E. coli*) والموجبة لجرام (*Staphylococcus aureus*) وبكتريوفاج F١١٦. دلت النتائج المتحصل عليها من إختبار نطاق التثبيط **inhibition zone** وإختبار نسبة البقاء **Survival percentage** على أن هذه المستخلصات النباتية تمتلك تأثير قوى مضاد للميكروبات، حيث وصلت نسبة القتل إلى ٩٨,١٤% فى البكتريا، ٩٢,٧% فى فاج F١١٦. بتقييم النشاط الطفرى بإستخدام بعض النظم الميكروبية وهى **Prophage induction, Transduction**، فى بعض المعاملات لم يلاحظ انطلاق أى جزيئات فاج من الخلايا الليسوجينية وأيضا لم تظهر خلايا **Transductants** مما يعكس أن المستخلصات النباتية ليس لها تأثير مطفر. وبذلك قادتنا هذه النتائج إلى دراسة التأثير المضاد للطفور لهذه المستخلصات، وقد أوضحت الدراسة أن المستخلصات النباتية الثلاثة المستخدمة فى هذه الدراسة لها نشاط مضاد للطفور ضد مادة إيثيل ميثيل سلفونات ذات التأثير الطفرى القوى.