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Studies on Antioxidant Potency and In Vitro Antibacterial Efficacy of *Mirabilis jalapa* on Enteric Pathogens

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Abstract

The present study aimed at analyzing the antibacterial efficacy of Mirabilis jalapa against different enteric bacteria includes Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella flexineri and Enterobacter aerogenes. Aqueous, Ethanolic, and hexane extracts of Mirabilis jalapa at the concentrations of 100, 200, 300, 400 and 500 mg were prepared for this study. The antioxidant properties of those extracts were also studied. Final results were statistically analyzed and tabulated.

Keywords: Antioxidant, Antibacterial, Enteric pathogens and Mirabilis jalapa

Introduction

Herbal medicine is frequently a part of a larger therapeutic system such as traditional and folk medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. The search and use of drugs and dietary supplements derived from plants have been accelerated in recent years. Ethno pharmacologist, botanist, microbiologist and natural product chemist are combing the medicinal flora for biological substances that could be developed for the treatment of infectious diseases.

Mirabilis jalapa, Family: Nyctaginaceae , It is a perennial herb or under shrub. An erect herb to about one meter high, native of Peru, but now dispersed throughout the tropics. The plant is decorative with red or white flowers and is a favorite garden plant surviving under conditions of neglect. English, French and some of the Africa. Leaf anti inflammatory, boils, root purgative, aphrodisiac, spasmolytic. Leaf juice used as an external application to wounds, bruises and for allaying itching in urticaria. Roots thickened and tuberous,upto 1m high ,stems swallon at nodes.leaves ovate, cordate, flowers in clusters , funnel- shaped , simple or double, fragrant, white, yellow, pink or purple nut ellipsoid, one seeded (Chopra et al,1980; The Wealth of India, 1962 and Dictionary of Indian Medicinal Plants, 1988)

The aim of the present investigation is to study the antibacterial effect of aqueous, ethanolic and hexane extract of *Mirabilis jalapa* against enteric pathogens and also the assessment of its antioxidant property.

Materials and Methods

Preparation of Plant extract

Mirabilis jalapa used in this study were collected from the college campus, Karpaga Vinayaga college of Engineering and Technology, Maduranthagam TamilNadu. A voucher specimen was deposited in our departmental laboratory. The collected plant sample was refluxed in running tape water for 1-2 h and shade dried at room temperature for 15 – 20 days. Aqueous, ethanolic and hexane extract of *Mirabilis jalapa* was prepared using soxhlet apparatus (Hoffman et al., 2004) for about 24h. The extract was distilled and concentrated *in vacuo* with addition of CaCl2. Lyophilized aqueous fractions were further used to test for the antibacterial and antioxidant properties.

Bacterial cultures

Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella flexineri and Enterobacter aerogenes were purchased from IMTECH, Chandigar, India. Solvent and other chemicals which were used during this study were from Himedia, Merck and s.d. Fine-Chemicals, Mumbai.

Antibacterial activity assessment

The antibacterial activity of *Mirabilis jalapa* was evaluated by agar well diffusion method (Chung *et al*., 1990). Muller Hinton agar medium was prepared and poured into the petridishes. Then it was inoculated with a swab of bacterial culture (mid log phase) and spread throughout the medium uniformly with a sterile cotton swab. Using a sterile cork borer (10mm diameter) wells were made in the agar medium. The test compound was introduced into the wells and all the plates were incubated at 37ºC for 24 h. The experiment was performed five times under strict aseptic conditions. Sensitivity of the organism was determined by measuring the diameter of the zone of inhibition. Each assay was repeated for five times and the mean value was taken for

analyses. The control experiment was carried out with antibiotics such as streptomycin and chloramphenicol (Table 1.1, 1.2 & 1.3).

Antioxidant activity assessment

The antioxidant activity of aqueous, ethanolic and hexane extracts of *Mirabilis jalapa* were determined by ferric thiocynate method (Mistuda et al., 1996). 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (100, 200, 300, 400 & 500µg/mL) were prepared. A mixture of a 2 mL of sample in 99.5% ethanol, 2.052 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05 M phosphate buffer (pH 7.0) and 1.948 mL of water was placed in a vial with a screw cap and placed in an oven at 60^{o}C in the dark. To 0.1 mL of this sample solution 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocynate was added. After the addition of 0.1 mL of 2 x 10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 3 min at 500 nm (Matook and Hashinaga, 2005). The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of α-tocopherol (reference compound) (Ali Yildirim et al., 2001). The inhibition of lipid peroxidation in percentage (Table 2.0) was calculated by following equation:

$$\% \text{ Inhibition} = 1 - (A1/A2) \times 100$$

Where,
A1 - absorbance of the test sample
A2 - absorbance control reaction

Results and Discussion

Table 1.1: Antibacterial activity of aqueous extract of *Mirabilis jalapa* against bacterial strains.

Aqueous extract	Concentration in µg	Diameter of the zone of inhibition (mm)				
		E.coli	*P.mirabilis*	*S.typhi*	*S.flexineri*	*E.aerogenes*
Mirabilis jalapa	100	11 ±0.4	NS	NI	NI	NI
	200	13 ±0.5	11 ±0.4	NS	NI	NS
	300	16 ±0.3	14 ±0.7	12 ±0.5	NS	NS
	400	20 ±0.6	17 ±0.8	14 ±0.3	21 ±0.2	14 ±0.8
	500	23 ±0.7	21 ±0.6	17 ±0.4	23 ±0.4	18 ±0.5
Streptomycin	100	25 ±0.2	22±0.8	20 ±0.3	27 ±0.6	23 ±0.4
Chloramphenical	100	26 ±0.2	22 ±0.4	21 ±0.7	28 ±0.3	22 ±0.8

NS – Non significant value (<10mm)
NI – No Inhibition

Table 1.2: Antibacterial activity of ethanolic extract of *Mirabilis jalapa* against bacterial strains.

Aqueous extract	Concentration in µg	Diameter of the zone of inhibition (mm)				
		E.coli	*P.mirabilis*	*S.typhi*	*S.flexineri*	*E.aerogenes*
Mirabilis jalapa	100	NS	NI	NI	NI	NS
	200	NS	NS	NS	NS	NS
	300	11 ±0.6	11 ±0.6	14 ±0.4	13 ±0.7	11 ±0.7
	400	15 ±0.6	16 ±0.8	16 ± 0.6	15 ±0.3	14 ±0.8
	500	18 ±0.3	19 ±0.2	18±0.4	18 ±0.4	18±0.5
Streptomycin	100	22 ±0.2	22±0.9	21 ±0.8	22 ±0.6	23 ±0.8
Chloramphenical	100	24 ±0.2	21 ±0.4	22 ±0.4	21±0.9	21 ±0.3

NS – Non significant value (<10mm)
NI – No Inhibition

Table 1.3: Antibacterial activity of hexane extract of *Mirabilis jalapa* against bacterial strains.

Aqueous extract	Concentration in µg	Diameter of the zone of inhibition (mm)				
		E.coli	*P.mirabilis*	*S.typhi*	*S.flexineri*	*E.aerogenes*
Mirabilis jalapa	100	NI	NI	NI	NI	NS
	200	NS	NI	NS	NS	NS
	300	NS	12 ±0.4	NS	11 ±0.7	NS
	400	13 ±0.2	15±0.7	14 ± 0.8	14 ±0.4	10 ±0.8
	500	16 ±0.3	18 ±0.3	16±0.5	16 ±0.7	12±0.5
Streptomycin	100	23 ±0.4	20±0.9	19 ±0.8	22 ±0.9	19 ±0.8
Chloramphenical	100	22 ±0.2	21 ±0.6	20 ±0.4	20±0.2	21 ±0.8

NS – Non significant value (<10mm)
NI – No Inhibition

Table 2.0 : Antioxidant activity of aqueous, ethanolic and hexane extracts of *Mirabilis jalapa.*

S.No	Extract	% of inhibition of lipid peroxidation				
		100µg/ml	200µg/ml	300µg/ml	400µg/ml	500µg/ml
1.	Water	19.68	27.45	39.87	46.45	60.23
2.	Ethanol	24.23	37.60	46.42	53.77	68.87
3.	Hexane	21.68	29.45	36.77	46.45	51.23
4.	α-Tocopherol	28.64	39.72	51.85	62.18	73.34

Antibacterial Activity

From Table 1.1, 1.2 & 1.3 it is very clear that the aqueous, ethanolic and hexane extracts of *Mirabilis jalapa* showed growth inhibition activity at the concentrations of 300mg to 500mg. *E.coli* and *S.flexineri.* were sensitive to aqueous extracts of *Mirabilis jalapa* at higher concentrations (400 and 500mg) rather than ethanolic and hexane extracts. *S.typi* and *E.aerogenes* were not highly susceptible to all extracts when compared to others (Table 1.1, 1.2 & 1.3). All the strains showed some resistance at mild concentrations (100 & 200mg) in all the three extracts. *P.mirabilis* showed moderate sensitivity only at the concentration of 500mg aqueous extract *Mirabilis jalapa*. It was observed that in both ethanolic and hexane extracts of *Mirabilis jalapa*, bacterial strains are not highly susceptible even at high concentration than aqueous extract.

Antioxidant Activity

The antioxidant activity of the aqueous, ethanolic and hexane extracts of *Mirabilis jalapa* were determined by ferric thiocynate (FTC) and the values are presented in Table 2.0. FTC method was used to determine the amount of peroxide formed and that react with ferrous chloride (FeCl2) to form a reddish ferric chloride (FeCl3) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity of extract increases. Aqueous, ethanolic and hexane extracts at various concentration (100,200, 300,400 and 500 in μg/mL), showed antioxidant activities in a concentration dependent manner. Ethanol extract at the concentration of 500 μg/mL showed 68.87%, an antioxidant activity at the concentration of 500 μg/mL of α-tocopherol (73.34%), the reference compound. The aqueous and hexane extracts of *Mirabilis jalapa* also have showed some significant level of inhibition of lipid peroxidation. It has been observed that the extract exhibited moderate antioxidant activity with the increase in concentration of the extract indicating that polyphenols or flavanoids may play important roles in the activities.

From literature review and phytochemical analysis it was found that the plant, *M. jalapa*, contains phenolic as well as flavonoid type compounds. Our present study results also reflected that aqueous, ethanolic and hexane extracts of *M.jalapa* showed significant antioxidant activity. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of oxidation (Owen et al., 2003). The biological property like antioxidant activity, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds (Harborne et al., 2000). From the above studies, it is clear that due to its polyphenolic and flavanoid like secondary metabolite content, *Mirabilis jalapa* may have antioxidant property.

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