Int. J. Chem. Sci.: 8(1), 2010, 559-564

IN VITRO ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS OF *MIRABILIS JALAPA* LEAVES

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ABSTRACT

The leaves of the plant *Mirabilis jalapa* were successively extracted with petroleum ether, benzene, chloroform, ethyl alcohol and methanol by Soxhlet extractor and water extract by cold maceration. Disc diffusion method was employed to determine the effect of antibacterial potential against Gram positive *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis,* gram negative *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and antifungal potential against *Candida albicans*. Methanol extract showed stronger and broader spectrum of microbial activity as compared to other extracts. Amikacin (10 µg/mL) drug was used as the standard antibacterial agent.

Key words: *Mirabilis jalapa*, Minimum inhibitory concentration, Bactericidal, Fungicidal, Amikacin.

INTRODUCTION

To a bacterium, the human body is a collection of environmental niches that provide the warmth, moisture, and food necessary for organism to grow. The bacteria have acquired genetic traits that enable them to enter the environment, remain in a niche, gain access to food sources, and escape clearance by host immune and non immune protective responses. Unfortunately, many of the mechanisms that bacteria use to maintain their niche and the by-products of bacterial growth are incompatible with the system of the human host. Many of these genetic traits are virulence factors, which enhance the ability of a bacterium to cause disease. Although most bacteria cause disease by directly destroying tissue, some release

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toxins, which are then disseminated by the blood to cause system-wide pathogenesis. Not all bacteria cause disease, but some always cause disease once infection occurs. The symptoms of a disease are determined by the function of the tissue affected, although systematic responses, produced by toxins and immune responses may also occur. The seriousness of the symptoms depends on the importance of the organ affected and the extent of the damage caused by the infection. The inoculums size is a major factor in determining whether disease occurs. However, this can vary from a relatively small inoculums (e.g. fewer than 200 *Shigella* for shigellosis) to a very large inoculums (e.g. 108 *Vibrio cholerae*)

Mirabilis jalapa, Family: Nyctaginaceae, is a perennial herb or undershrub. 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 in England, France and some of the Africa. Leaf is anti-inflammatory, boils, root purgative, aphrodisiac and spasmolytic. Leaf juice is used as an external application to wounds, bruises and for allaying itching in urticaria. Roots thickened and tuberous, upto 1 m 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.[1-4]

EXPERIMENTAL

Materials and methods

Plant material

Mirabilis jalapa was collected in Nagerkovil Dist of Tamilnadu. The voucher specimen has been deposited at the museum of the Dept. of Pharmacognosy, K. M. College of Pharmacy, Madurai and authenticated by a taxonomist.

Preparation of extracts

The shade dried and powdered leaves were extracted successively with petroleum ether, benzene, chloroform, ethanol and methanol by Soxhlet extractor and water extract by cold maceration.

Preliminary phytochemical investigation[5-8]

The qualitative chemical test of various extracts of *Mirabilis jalapa* was carried out using standard procedure showed the presence of glycosides, saponins, tannins.

Micro-organisms used

Gram - positive bacteria: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis*,

Gram - negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*,

Fungi: *Candida albicans.*

Antimicrobial activity by disc diffusion assay[9-11]

Various extracts were dissolved in the same solvent to a final concentration of 30 mg/mL and sterilized. Antimicrobial tests were then carried out by disc-diffusion method. The density of the bacterial suspension was standardized by using Mac Farland standard method. The discs were impregnated with 10 µL of the extracts (300/disc) at the concentration of 30 mg/mL and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ciprofloxacin 10 µg/disc and amphotericin B 100 units/disc were used as positive reference standards. The inoculated plates were incubated at 37 ^{0}C bacteria and 25 ^{0}C for fungi[12].

Micro dilution assay[12,13]

The minimal inhibition concentration values were also studied for the microorganisms in disc-diffusion assay. The inoculums prepared from 12-hour broth cultures and suspensions were adjusted to 0.5 Mc Farland standard turbidity. The *Mirabilis jalapa* extract dissolved in 10% dimethyl sulfoxide were first diluted to the highest concentration (500 µg/mL) to be tested, and then serial two fold dilutions were made in a concentration range from 7.8-500 µg/mL. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *Mirabilis jalapa* extract against bacteria and minimum fungicidal concentration against *C. albicans* were determined based on a micro-well dilution method.

Disc diffusion assay

The antimicrobial activity of *Mirabilis jalapa* leaves extracts against micro-organisms examined in the present study and their potency were quantitatively assessed by the presence or absence of inhibition zones and zone diameters, minimum inhibitory concentration and minimum bactericidal concentration values. All the extracts at concentration of 300 µg/mL inhibited the growth of *C. albicans*. All extracts had inhibitory activity against gram positive and gram negative bacteria (Table 1) except petroleum ether extract.

Minimum inhibitory concentration

MIC of *Mirabilis jalapa* was as low as 18 µg/mL against *E. coli*. This concentration was at 27 µg/mL for *Bacillus subtilis*, 33 µg/mL for *Klebsiella pneumoniae*, *Mirabilis jalapa* at concentration of 20 µg/mL inhibited the growth of *Staphylococcus aureus, Staphylococcus epidermis* 21 µg/mL, *Pseudomonas aeruginosa* 27 µg/mL and 30 µg/mL for *C.albicans*.

Minimum bactericidal and fungicidal concentration

The bactericidal and fungicidal maximum concentration of *Mirabilis jalapa* is 37 µg/mL for *Staphylococcus aureus,* 39 µg/mL *Staphylococcus epidermis, Pseudomonas aeruginosa* 36 µg/mL, 36 µg/mL for *C. albicans,* 33 µg/mL for *Bacillus subtilis*, *Klebsiella pneumoniae* 36 µg/mL and for *E.coli* 35 µg/mL. MBC, MFC and MIC concentrations are reported in mg/mL.

RESULTS AND DISCUSSION

Results reveal that various extracts of *Mirabilis jalapa* leaves were significantly effective against gram positive & gram negative bacteria fungi, where as significant activity was not observed with petroleum ether extract (Table 1). Minimum inhibitory and minimum bactericidal concentration of the active extracts are shown in Table 2. Preliminary phytoconstituents screening of the extracts showed the presence of glycoside, flavonoids, tannins and saponins. Thus, further work can be carried out on the isolation. In addition, these results confirmed the evidence in previous studies, which reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents.[15-17]

Table 1: **Antimicrobial activity of *Mirabilis jalapa* leaves of various extracts against the bacterial and fungal strains tested based on disc-diffusion method**

Plant extract	Zone of inhibition (mm in diameter) at conc. 300 µg/disc				
Micro organisms	**Pet. ether**	**Chloroform**	**Ethanol**	**Methanol**	**Aqueous**
Staphylococcu aureus	-	17	21	25	23
Staphylococcus epidermidis	-	16	19	22	20

Cont…

Plant extract	Zone of inhibition (mm in diameter) at conc. 300 µg/disc				
Micro organisms	**Pet. ether**	**Chloroform**	**Ethanol**	**Methanol**	**Aqueous**
Bacillus subtilis	-	16	19	21	20
Pseudomonas aeruginosa	-	16	20	21	23
Klebsiella pneumoniae	-	16	15	20	21
Escherichia coli	-	18	19	22	20
Candida albicans	-	13	13	23	16

Antibiotic disc: Bacteria –Amikacin 10 µg/disc. *C. albicans* – Amphotericin B 100 units values are expressed as mean ± S.

Table 2: The MBC and MIC values of *Mirabilis jalapa* leaves of various extracts against the bacterial and fungal strains tested in micro dilution assay

Micro organism	Chloroform		Methanol		Aqueous	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus	29	31	33	37	20	28
Staphylococcus epidermidis	21	30	34	39	31	34
Bacillus subtilis	30	24	27	33	32	30
Pseudomonas aeruginosa	27	31	31	36	29	32
Klebsiella pneumoniae	32	29	33	36	33	32
Escherichia coli	33	29	32	35	18	30
Candida albicans	30	30	33	36	20	29

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Accepted : 14.10.2009