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RESEARCH ARTICLE

IN VITRO EFFECT OF PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF **MIRABILIS JALAPA**

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ARTICLE INFO	ABSTRACT		
<i>Article History:</i> Received 18 th March, 2012 Received in revised form 20 th April, 2012 Accepted 28 th May, 2012 Published online 30 th June, 2012	The present study was aimed to identify the antibacterial activities of <i>Mirabilis jalapa</i> chloroform extract against bacterial and fungal pathogens viz., <i>Escherichia coli</i> , <i>S. epidermidis</i> , <i>K. pneumonia</i> , <i>C. freunndii</i> , <i>S. marcescens</i> , <i>Proteus vulgaris</i> , <i>Bacillus subtilis</i> , <i>S. aureus</i> , <i>Candida albicans</i> and <i>Cryptococcus sp.</i> In this study, the maximum zone of inhibition was identified with <i>Escherichia coli</i> ; this was followed by <i>S. marcescens</i> , <i>Bacillus subtilis</i> and <i>K. pneumonia</i> . The preliminary phytochemical analysis showed the presence of flavanoids, saponins, terpenoids, tannin & phenols,		
Key words:	carbohydrate and amino acid compounds with chloroform extract. It is concluded from the present findings that, the chloroform extract of <i>M. jalapa</i> can be used as potential antibacterial agents after		
<i>Mirabilis jalapa</i> , Antibacterial activity, preliminary phytochemical, MBC,	completion of clinical trials.		
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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (Nagri et al., 1991). Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine (Raja et al., 2010). The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Gnanadesigan et al., 2011). Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Astal et al., 2005). In view of this, the present study was aimed to identify the antibacterial potential of Mirabilis jalapa chloroform extract against some infectious pathogens.

MATERIALS AND METHODS

Collection and Identification of M. jalapa

The whole plant material was collected from Kolli Hills, Namakkal district, Tamil Nadu, India. The collected plant materials were washed thrice in tap water and twice with distilled water to remove the adherent contaminants and dust

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particles. Voucher specimen was (SASMP0065) deposited at the Department of Microbiology, Selvamm Arts and Science College, Namakkal, TamilNadu. The collected sample was authenticated by Mr. P. Subramaniam Asst. Professor and Taxonomist, Aringar Anna Government Arts College, Namakkal, Tamilnadu, India.

Extraction of secondary metabolites

The collected samples were shade dried at room temperature. After completion of drying the plant material was coarse powdered with mechanical motor. The powdered plant material was soxhalted with chloroform solvent for 72 hrs. The extract was concentrated under in vacuum under rotary phase evaporator. The preliminary phytochemical analysis was performed with standard protocols (Ravikumar et al., 2010b)

In vitro antimicrobial activity

Test organisms

Test bacterial pathogens such as Escherichia coli- MTCC 443, S. epidermidis- MTCC 435, K. pneumonia- MTCC 3384, C. freunndii- MTCC 1658, S. marcescens- MTCC 97, Proteus vulgaris- MTCC 426 and Bacillus subtilis- MTCC 2620 were collected from Microbial Culture Collection Center (MTCC, Chandigah) and the cultures were gowned with their respective medium compositions, Further, the microbial cultures such as S. aureus, Candida albicans and Cryptococcus sp. was procured from PG Research Department of Microbiology, Vivekananda College, Tiruchengode, Tamilnadu, India.

Disc diffusion assay

The antibacterial activity was performed with disc diffusion assay with standard protocols (Ravikumar *et al.*, 2010a). In brief, 500 µg.disc⁻¹ of plant extract was impregnated into whattmann filter paper no.1 (6mm. dia). Overnight broth cultures of different bacterial pathogens were swabbed on to the molten sterile Mueller Hinton agar plate. After that, the extract impregnated discs were placed in to the corresponding petriplates. The plates were incubated at 37°C for 24 hrs. The antibacterial activity was measured with the zone of inhibition formed around the discs. Control plates were also maintained without the addition of solvents.

Minimum Inhibitory Concentration (MIC)

500µl of various concentrations (1000, 500, 250, 125, 62.5, 32.125, 16.06, 8.03 µg.ml⁻¹) of extract stock solutions were mixed with 500µl of nutrient broth and 50 µl of bacterial pathogens suspensions individually. Nutrient broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 24 h. The MIC was the lowest concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the basis of turbidity (Ravikumar *et al.*, 2010b).

Minimum Bactericidal Concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 ml loop and incubated at 37° C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media (Ravikumar *et al.*, 2010b)

RESULTS AND DISCUSSION

The results of the preliminary phytochemical analysis showed the presence of flavonoids, saponins, terpenoids, tannins and phenols and the steroids and alkaloids (Table 1). Further, the disc diffusion assay showed the maximum zone of inhibition with *Escherichia coli* (15.5±0.70 mm) followed by *S. marcescens* (15.09±0.62 mm), *Bacillus subtilis* (14.62±0.82) and *K. pneumonia* (12.73±0.62). Similarly, the result of MIC and MBC values were varied between 125- 1000 µg. ml⁻¹ (Table 2).

 Table 1. Preliminary phytochemical analysis in M. jalapa

 chloroform extract

Name of the phytochemical	Chloroform extract	
Alkaloids	-	
Flavanoids	+	
Steroids	-	
Saponins	+	
Terpenoids	+	
Tannin & phenols	+	
Carbohydrate	+	
Amino acid	+	

 Table 2. Antimicrobial activities, MIC and MBC in *M. jalapa*

 chloroform extracts against different pathogens

Name of the phytochemical	Disc diffusion assay (mm)	$MIC \ (\mu g.m^{-1})$	MBC (µg.ml ⁻¹)
Escherichia coli	15.5±0.70	500	1000
S. epidermitis	-	-	-
K. pneumonia	12.73±0.62	125	500
C. freunndii	-	-	-
S. marcescens	15.09±0.62	500	1000
Proteus vulgaris	-	-	-
Bacillus subtilis	14.62 ± 0.82	500	1000
S. aureus	-	-	-
Candida albicans	-	-	-
Cryptococcus sp.	-	-	-

The presence of antibacterial substances in the higher plants is well established (Ravikumar et al., 2011a). The inhibitory activities exhibited by the tested plants tends to agree with the report that antibacterial properties of these plants are due to the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils (Atindehou et al., 2002 and Fennel et al., 2004). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Nair et al., 2005). In this connection, one such plant named M. jalapa was selected for antimicrobial potentiality. Ravikumar et al. (2010c) noted, the chloroform extract showed broad spectrum of antibacterial activities against Gram positive and Gram negative bacterial cells, the antibacterial potential of the chloroform extract might be due to the presence of unique phytochemical constituents in the plant extract. Further, the antibacterial activity was found higher in Gram negative bacterial cells than Gram positive and this might be due to the impermeable nature of multi layered outer peptidoglycon layer in gram positive cells and permeable nature of outer phospholipidic membrane in Gram negative bacterial cells (Ravikumar et al., 2010c). In some instances, the values of MBC was found higher than the MIC values and this might be due to the endospore formation, which are resistant to conditions to which vegetative cells are intolerant (Ravikumar et al., 2010b). Similar, reports are also identified with several plant extracts (Edeoga et al., 2005; Rojas et al., 2003). Further, the preliminary phytochemical analysis showed the presence of heterogeneous mixture of secondary metabolites and the antibacterial activity of the metabolites can be provoked as synergistic or antagonistic manner (Ravikumar et al., 2010a). It is concluded from the present findings that, the chloroform extract of M. jalapa can be used as potential antibacterial agents. Further, the effectiveness of the chloroform extract with other bacterial pathogens is in progress.

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