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Phytochemical Screening and Antimicrobial Activity of the Leaf Extract of *Mirabilis jalapa* Against Pathogenic Microorganisms

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Abstract

Investigation of the phytochemical constituents and antimicrobial activity of the leaf extracts of *Mirabilis jalapa* were carried out using acetone, chloroform, ethanol and methanol. These extracts were subjected to screening of preliminary phytochemical tests. Phytochemical analysis showed the presence of alkaloids, flavanoids, phenols, glycosides, tannins, saponins and lignins. The methanol extract exhibited the largest zone of inhibition (21mm in dia with 500µg/disc extract) against *Staphylococcus aureus* and the highest inhibition of fungal radial mycelial growth (97.5% with 500µg/ml medium) against *Aspergillus flavus*. The methanol extract exhibited the lowest MIC against *Staphylococcus aureus* (39 µg/ml) and *Aspergillus flavus* (45µg/ml). It appeared that M. jalapa could be a potential natural source of new antimicrobial agent.

Keywords: *Mirabilis jalapa*, leaf extract, phytochemicals, antimicrobial activity.

Introduction

Natural products perform various functions, and many of them have interesting and useful biological activities [1]. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Most of the people in rural and urban areas of the world were depend on the medicinal plants for the treatment of infectious diseases. Over one and a half million practitioners of the Indian System of Medicine in the oral and Codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity [2-5].

Mirabilis jalapa belongs to the family Nyctaginaceae plant has been referred with the name as the four 0'clock flower plant. It 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. This plant has a history of use for the treatment of various ailments and the most

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commonly used plant part for this purpose is the root, bark and leaves can also be utilized. Leaf juice used as an external application to wounds, bruises and for allaying itching in urticaria. Several research works have been carried out to study about the phytochemical components of *Mirabilis jalapa* and also about the antimicrobial activity of the plant [6, 7]. This study was therefore designed to investigate the phytoconstituents present in this plant and to determine its antimicrobial activity against some bacterial and fungal pathogens.

Materials and Methods

Collection of Plant Samples

The leaves of the plant *Mirabilis jalapa* were collected from the plants grown in S. V. University Campus. The taxonomic identities of plants were confirmed by Dr. Yashodamma, Taxonomist, Department of Botany, S. V. University, Tirupati, India for the Botanical verification and authenticating the plant material.

Test Microorganisms

Four bacterial and four fungal species were employed as test organisms. These include:, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Streptococci pneumonia, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus terreus*, which were obtained as fresh pure cultures from the Department of Microbilogy, Sri Venkateswara University, Tirupati, India. The fungal and bacterial strains were maintained on PDA and Mueller-Hinton agar slants in the refrigerator at 4^0C prior to use.

Preparation of Extracts

Collected fresh leaves of *Mirabilis jalapa* were washed thoroughly in running tap water to remove debris and dust particles and then rinsed in sterile distilled water, shade dried at room temperature, weighed (100g) and ground in a sterile mortar. The powdered material was then kept in air tight containers until use. For extraction of bioactive components 20g each of the powdered samples were soaked into 100ml of acetone, chloroform, ethanol and methanol for 72 h with stirring at 24 h interval. The extracts thus obtained were filtered, centrifuged at 5000 rpm for 20 minutes and then concentrated to a gummy material under reduced pressure at 50^0C by rotary vacume evaporator. The extracts were then transferred to small vials and the storage of the extracts took place in the refrigerator.

Preliminary Phytochemical Analysis

The freshly prepared leaf extracts were subjected to standard analysis for the presence of phytoconstituents as described by Horborne [8].

Antibacterial Assay

Antibacterial activity was determined against four pathogens by disc diffusion method [9]. The isolated compound was dissolved in appropriate solvent. Different concentration of acetone, chloroform, ethanol and methanol extracts of *Mirabilis jalapa* were tested against pathogenic bacteria. The test micro organisms were seeded into respective medium by spread plate method 100µl (10^6 cells/ml suspension) with the 24 hours cultures of bacteria grown in nutrient agar medium. Petridishes (measuring 90mm diameter) containing 15-20 ml of nutrient agar medium. After solidification Whatman No: 1 paper discs (5mm in diameter) impregnated with the extracts was placed on test organism-seeded plates. Streptomycin sulphate (10µg/ml) used as the reference antibacterial agent. The antibacterial assay plates were incubated at 37^0C for 24 hours. After incubation period, the diameter of inhibition zone was measured in mm. All the experiments were performed in triplicates and average diameter zone of inhibition was recorded. An inhibition zone of 10mm or greater (including diameter of the disc) was considered antibacterial activity. The Minimum Inhibitory Concentrations (MICs) for the most active component were recorded after 24 hours.

Antifungal Activity

The antifungal activity was evaluated following the method described by Miah et al. [10] with

slight modifications in the volume and concentrations of extracts and mycelial disc size used. Briefly, 0.5 ml of various concentrations (100-500 µg/ml) of extracts were dispensed in different sets of sterilized potato dextrose agar were added, swirled to achieve a uniform mix and allowed on the bench for 30 min to solidify in the sterile environmental conditions. Thereafter, a mycelial disc of 5mm in diameter for each of the filamentous fungi with the help of 5mm sterile cork borer from the periphery of a 7 days old culture, was inoculated in the centre of each petriplate, and then incubated at 27 ± 1^0C for 5-7 days. Flucunazole mixed with medium served as the reference antifungal agent. The diameter of growth of the hypha were measured on 5th day after inoculation, and the percentage of inhibition was calculated by using the following formula

$$\% \text{ inhibition} = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

The Minimum Inhibitory Concentrations (MICs) for the most active component were recorded after 72 hours.

Results and Discussion

The preliminary phytochemical screening of M. jalapa extract showed the presence of bioactive components like alkaloids, flavonoids, phenols, glycosides, tannins, saponins and lignin (Table 1). The plants are the vital source of innumerable numbers of antimicrobial compounds. Several phytoconstituents like flavanoids [11], tannins [12], saponins and polyphenols [13] are effective antimicrobial substances against a wide range of microorganisms.

Table 1: Phytochemical components of leaf extract of *Mirabilis jalapa*

Phytochemical components	Leaf extract
Alkaloids	+
Flayanoids	+
Steroids	-
Phenols	+
Glycosides	+
Tennins	+
Saponins	+
Volatile oils	-
Lignins	+

+ : Present
- : Absent

Antibacterial Studies

Acetone, chloroform, ethanol and methanol extracts of *M. jalapa* were investigated for their antibacterial activity was tested against pathogenic bacteria at different concentrations of 100, 300 and 500µg/disc and results have been illustrated (Table 2). The growth of *B. subtilis*, *E. coli*, *S. aureus* and *S. pneumonia* was inhibited considerably particularly at the higher dose (500µg/disc). The highest growth of inhibition were recorded in *S. aureus* in methanol extract (21.0mm) followed by *E. coli* (19.5mm), *S. pneumonia* (19.0mm) and *B. subtilis* (18.5mm). similarly, in chloroform extracts, the maximum zone of inhibition were observed in *S. aureus* (18.5mm) followed by *B. subtilis* (18.0mm), *S. pneumonia* (17.5mm) and *E. coli* (14.7mm).

Table 2: Antibacterial activity of leaf extract of *M. jalapa* against pathogenic bacteria

Pathogenic Bacteria	Streptomycin sulphate (10µg/ml	Extracts (Zone of inhibition in mm)											
		Acetone			Chloroform			Ethanol			Methanol		
		100	300	500	100	300	500	100	300	500	100	300	500
B. subtilis	17.5	7.1	9.5	11.6	8.3	11.5	18.0	7.1	9.5	11.5	8.5	13.2	18.5
E. coli	16.1	8.	10.6	13.5	9.2	12.8	14.7	7.5	10.5	12.8	10.1	13.8	19.5
S. aureus	18.5	-	8.3	10.2	8.7	11.5	18.5		8.3	10.0	10.5	13.7	21.0
S. pneumonia	15.3	-	8.5	10.8	8.3	11.0	17.2		7.5	8.3	9.5	13.3	19.0

*Each value is a mean of two replicates
- ; No zone inhibition

Table 3: MIC of leaf extract of *M. jalapa* against pathogenic bacteria

Bacterial Strains	MIC (µg/ml disc)				
	Streptomycin sulphate	Acetone	Chloroform	Ethanol	Methanol
B. subtilis	7.0	95	43	93	49.5
E. coli	8.5	93	43	91	41
S. aureus	6.5	42.5	197	195	39
S. pneumonia	9.0	193	43	295	45

The acetone extracts exhibited maximum zone of inhibition were observed in *E. coli* followed by *B. subtilis, S. pneumonia* and *S. aureus*. In ethanol extracts growth of inhibition against *B. subtilis, E. coli, S. aureus* and *S. pneumonia* were 11.5mm, 12.8mm, 10.0mm and 8.3mm respectively at 500µg/disc. Antibacterial antibiotic streptomycin sulphate (10µg/disc) was also found to be active against all the bacteria tested herein. The methanol extract exhibited the lowest MIC value (39µg/ml) against *S. aureus* (Table 3). Similar antibacterial activity of some other plant extracts has been reported previously [14-16]. These results indicate the presence of high amount of phytoconstituents in the organic plant extracts are the responsible for the antimicrobial activity.

Table 4: Antifungal activity of leaf extract of M. jalapa against pathogenic fungi.

Pathogenic Fungi	Flucunazole (100µg/ml)	Extracts (%inhibition of fungal radial growth in cm)											
		Acetone			Chloroform			Ethanol			Methanol		
		100	300	500	100	300	500	100	300	500	100	300	500
A. flavus	84.0	23.6	52.0	83.0	28.3	63.3	91.0	11.5	50.4	79.5	30.5	79.7	97.5
A. fumigatus	87.6	15.3	58.2	81.2	31.4	67.3	93.5	12.7	51.5	81.0	34.5	70.5	95.0
A. niger	92.4	17.3	49.5	80.0	38.5	76.0	95.5	21.0	53.0	81.0	29.5	69.3	91.0
A. terreus	93.0	25.0	74.3	89.7	33.0	71.5	92.5	17.0	47.0	78.3	31.0	73.0	95.3

*Each value is a mean of two replicates

Table 5: MIC of leaf extract of *M. jalapa* against pathogenic fungi

Fungal Strains	MIC (µg/ml medium)				
	Flucunazole	Acetone	Chloroform	Ethanol	Methanol
A. flavus	42	92	52	95	45
A. fumigatus	41	93	49	90	47
A. niger	25	67	51	49	46
A. terreus	35	63	53	95	49

Antifungal Studies

The organic extracts i.e., acetone, chloroform, ethanol and methanol extracts have moderate to significant activity at the concentrations of 100, 300 and 500µg/ml medium against *Aspergillus flavus, A. fumigatus, A. niger* and *A. terreus* (Table 4). It appeared that the organic extracts inhibited the radial mycelial growth of all the test fungi at different concentrations of 100, 300 and 500µg/ml medium to varying degrees. The highest inhibition (97.5%) of fungal radial mycelial growth was recorded against *A. flavus* at a concentration of 500µg/ml medium using the methanol. Antifungal antibiotic flucunazole (100µg/ml medium) exhibited good inhibition of radial mycelial growth of all the four fungi tested herein, but it was much less active against *A. flavus* and *A. fumigatus* compared to that of the methanol and chloroform extracts. The lowest MIC (45µg/ml) was recorded against *A. flavus* with methanol extract (Table 5). Similar antifungal activities on plant extracts of other plants have also been previously reported [17, 18].

Conclusion

The antimicrobial activity of *M. jalapa* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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References

1. Galal M, Bashir AK, Salih AM, Adam SEI. Activity of water extracts of *Albizia anthelmintica* and *A. lebbek* barks against experimental *Hymenolepis diminuta* infection in rats. J. Ethnopharmacol, 1991; 31: 333-337.
2. Ahmed AMA, Rahman MS, Anwar MN. Antimicrobial activity of extracts and crude alkaloids isolated from leaf of *Adhathoda vasica* Nees. Bangladesh J Life Sci. 2002; 15(2): 125 – 128.
3. Krishnaraju AV, Rao VN, Sundararaju D, Vanisree M, Subbaraju GV. Assessment of bioactivity of Indian medicinal plant using brine shrimp lethality assay. Int. J. Appl. and Eng. 2005; 3(2): 125-134.
4. Ravikumar, Patil HS, Makari HK, Gurumurthy H. In vitro antimicrobial activity of ethanol extract of *Thevetia peruviana*. Electronic J Environ. Agri. Food Chem., 2007; 6(9): 2318-2322.
5. Pratima Mathad, Sundar SM. Phytochemical and antimicrobial activity of *Digera muricata* L Mart. E – Journal of Chemistry. 2010; 7(1): 275 – 280.
6. Muthumani P, Devi P, Meera R, Kameshwari, B, Eswarapriya B. In vitro antimicrobial activity of various extracts of *Mirabilis jalapa* leaves. The Internet Journal of Microbiology, 2009; 7(2): 120-124.
7. Ravi Sankar N, Sreeramulu A. In vitro efficacy of nineteen medicinal plant extracts on Uncinula tectonae, the powdery mildew of teak. J Biomed. 2009; 3(2): 137-141.
8. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edition. 1998; Chamman of Hall Pub. London, UK.
9. Ergene A, Guler P, Tan S, Mirici S, Hamzaoglu E, Duran A. Antibacterial activity and antifungal activity of *Heracleum sphondylium* sub sp. artivinense. Afri. J Biotechnology, 2006; 5(11): 1087-1089.

10. Miah MAT, Ahmed HU, Sharma NR, Ali A, Miah SA. Antifungal activity of some plant extracts. Bangladesh J Bot. 1990; 19(1): 5-10.
11. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant *Staphylococcus aureus*. Journal of Ethnopharmacol. 1996; 50: 27-34.
12. Ya C, Gaffney SH, Lilley TH, Haslam E. Carbohydrate-polyphenol complexation. P.553. In: Hemingway RW, Karchesy JJ (ed), 1988, Chemistry and Significance of condensed tannins. Plenum Press, New York.
13. Nabila B, Mirici S, Duran A. Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afri. J Pharmacol., 2008; 2(2): 22-28.
14. Rahman MS, Begum J, Chowdhury JU and Anwar MN Antimicrobial activity of *Holarrhena antidysenterica* against *Salmonella typhi*. Chittagong Univ. J. Sci. 1998; 22(1): 111-112.
15. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agri. Sci., 2008; 4: 839-843.
16. Gandhiraja N, Sriram, Meena V, Kavitha C, Sasikumar C, Rajeswari R. Phytochemical screening and antimicrobial activity of the plant extracts of Mimosa pudica L. against the selected microbes. Ethanobotanical Leaflets. 2009; 13: 618-624.
17. Anwar MN, Singha PJ, Chowdary JU. Antifungal activity of some plant extracts on phytopathogenic fungi. Bangladesh J. Life Sci. 1994; 6(2): 23-26.
18. Doughari JH, Obidah JS. In Vitro antifungal activity of stem bark extracts of *Leptadenia lancifolia*. Int. J Integrative Biology, 2008; 3(2): 111-117.