Indian Journal of Pharmacy and Pharmacology 2020;7(4):226–229

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

Antibacterial effect of ethanolic extract of *Mirabilis jalapa* (Alsaa Arbaa) seeds against some bacteria

Abdulrahman M A Saeed[1,*], Kawther A F Mahdi[2]

[1]*Dept. of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan*
[2]*Ministry of Animal Resources, South Darfur State, Sudan*

ARTICLE INFO

Article history:
Received 26-11-2020
Accepted 01-12-2020
Available online 25-01-2021

Keywords:
Mirabilis jalapa
Seeds
Staphylococcus aureus
Escherichia coli
Disc diffusion
Well diffusion

ABSTRACT

An indigenous medicinal plant *Mirabilis jalapa* (family: *Nyctaginaceae*) *was* widely used as a traditional medicine in many parts of the world for treatment of various diseases: antiviral, antibacterial, antifungal, anti tumours, anti-nociceptive, anti-inflammatory agent, laxative, dysentery, muscular pain and abdominal colic.

The research indicates the effectiveness of the seeds of 4-O'clock plant against *Staphylococcus aureus* and *Escherichia coli.* The seeds of the plant were collected, air dried, powdered and (100 gm.) subjected to gradient extraction using soxhlate apparatus. Ethanol 70% was used as a solvent. Three concentrations presenting 50% (500 mg/ml), 25% (250 mg/ml) and 12.5% (125 mg/ml) were prepared. *In-vitro* antibacterial assay of the ethanolic extract of the plant seeds was performed using agar wells diffusion method, disc diffusion method and dilution method with distilled water as negative control.

The seeds of the plant exhibited significant antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*. The greatest zone of inhibition was displayed by well diffusion method against *Staphylococcus aureus* at 500 mg/ml was 29 mm, at 250 mg/ml was 18 mm and at 125 mg/ml was 12 mm. But at same concentrations when were used disc diffusion method the result were displayed are 24 mm at 500 mg/kg, 9mm at 250 mg/ml and 7 mm at 125 mg/ml against *Staphylococcus aureus*. And 20 mm at 500 mg/ml, 14.3 mm at 250 mg/ml and 8.3 mm at 125 mg/ml against *Escherichia coli*.

1. Introduction

Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry (Preethi, et al., 2010). Numerous studies made throughout the world have demonstrated wide occurrence of antimicrobial compounds in higher plants (Joseph and Singh, 2008).[1] Studies on natural product are aimed to establish medicinal value of plant by exploration of existing scientific knowledge, traditional uses and discovery of potential therapeutic agents (Ramesh, and Mahalakshmi, 2013; 2014).[2,3]

Due to indiscriminate use of antibiotics, resistant pathogenic microorganisms have increased in recent time that has caused many clinical problems for the treatment of several infectious diseases (Harsha, et al; 2011).[4] Antimicrobial resistance to antimicrobial agents has lead to treatment failure and the shift of medical care from orthodox to herbal medicine (O

O-Sunday, et al 2008)[5] Due to emergence of drug resistant strains of pathogenic bacteria, it has become important to investigate plants as sources of novel antimicrobials, as they may inhibit bacteria by a mechanism different than that of currently used antibiotics. (Tomoko, et al; 2002).[6] Commonly used synthetic antibiotics create adverse effects in human. Hence research to develop new antimicrobial agents is urgently needed (Harsha, et al;

* *Corresponding author.*
E-mail address: dr.amasaeed@yahoo.com (A. M. A. Saeed).

https://doi.org/10.18231/j.ijpp.2020.037

2011).[4] This development has led to increased search to unfold new, broad spectrum, potent antimicrobial agents. Most of the herbal medicines in use await validation of their claimed effects and possibly the development of novel antimicrobial drugs from them (Otmenyn O-Sunday, et al; 2008).[5] Natural plants derived compounds contribute a lot in fight against pathogens. Various plant extracts have also been examined for their antibacterial activity with the objective of exploring environmentally safe alternatives of plant disease control (Gracelin, 2011).[7]

Most of these isolation were based on the utilization of such natural agents in traditional or folklore medicine. In human health care, this plant-based traditional medicine continue to play an important role. Nearly 80% of the people throughout the world rely on plant based medicine (Kensa, 2011).[8] Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have in-vitro antimicrobial properties (Edeoga, et al., 2005).[9] Demand for plants having medicinal value is increasing day by day in both developing and developed countries due to growing recognition of natural products as they are non narcotic, cost-effective and have no side effects. The present study investigate antibacterial activity in the seeds of one species of family Nyctagenaceae from the plant kingdom. It is commonly known as the Four o′clock family, as most of the species have flower that open in late afternoon or early evening (Levin, et al, 2001),[10] it is extraction has antibacterial, antiviral and antifungal activities (Oladummoye, 2007),[11] and it is used in traditional medicine in different countries for the treatment of diarrhea, dysentry, cojunctivitis, edema, inflmmation, swelling, muscular pain and abominal colics. (Daniel, 2006 and Holdsworth, 1992).[12]

This study was designed to investigate antibacterial effect of *Mirabilis jalapa*seeds against two bacterial species: *Staphylococcus aureus* and *Escherichia coli.*

2. Materials and Methods

Seeds of *Mirabilis jalapa* plant (Four O'clock plant) were collected in plastic cubs from Nyala area South DarFur. The plant was authenticated by Botanist of National Center for Research Medicinal and Aromatic plant Research Institute, Khartoum, Sudan.

2.1. The ethanolic extract of the seeds

Extraction was carried out according to method described by Sukhdev et al. (2008).[13] Hundred grams of dry seeds were coarsely powdered using mortar and pestle. Coarsely sample was successively extracted with 70% ethanol using soxhelt extractor apparatus(Duran UK). Extraction carried out for about six hours till the colour of solvent at the last siphoning time returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus (Buchi, Switzerland). Finally, extracts allowed to dry in Petri dish till complete dryness.

2.2. Plant extract concentrations

Concentrations presenting 50% (500 mg/ml), 25% (250 mg/ml) and 12.5% (125 mg/ml) were prepared.

- Weight of extract 5g was dissolved in 5 ml distilled water to make 100%.
- 2.5 ml of 100% was added to 2.5 ml distilled water to make 50% extract concentration.
- 2.5 ml of 50% was added to 2.5 ml distilled water to make 25%.
- 2.5 ml of 25% was added to 2.5 ml distilled water to make 12.5%.

2.3. Test organisms

The bacterial isolates used in this study were *Staphylococcus aureus* and *Escherichia coli.* These isolates were obtained from the stock of Department of Bacteriology, Central Veterinary Research Laboratory (CVRL), Soba, Khartoum.

2.4. Antibacterial activity

Antibacterial was determined using the agar diffusion methods. Three concentrations of *M. jalaba* extract were used (12.5%, 25%, 50%) in all methods.

2.5. Disc diffusion method

The tested organism was suspended was flooded onto agar plate and left for 10 minutes, the excessive solution was aspirated completly. Three filter paper disc (5mm diameter) each containing one of the tested dilution of the plant extract (50%, 25%, 12.5%) were placed onto the plate uniformally seeded with the tested organism and one disc embedded in distilled water was placed as control. This procedure was performed under strict aseptic condition. Three replicates were done. Then the plates were incubated at 37°C for 24 hr. The diameter of each zone of inhibition was measured in millimeters.

2.6. Wells diffusion method

0.2 ml of nutrient broth culture of the tested organism was added to 20 ml of sterile nutrient broth medium, then shacked well and the mixture was poured on to the petri dish and left to 10 minutes then for drying. Four wells (6 mm diameter) was made in the agar. 0.2 ml of the extract from each prepared concentrate was carefully dispensed into the well, and the plates were incubated for 24 hr at 37°C. This procedure was performed under aseptic condition. Three replicates were done and the diameter of each zone of inhibition was measured.

2.7. Broth dilution method

1. 1.8 ml nutrient broth was used as diluent.
2. 0.2 ml of bacterial culture of the tested organisms was added to give 2.0 ml culture conc. of the culture**

Two ml of different extract concentration (50%, 25%, 12.5%) was added to the bacterial suspension, incubated at 37̊ C aerobically for up to 48 hours and examined for bacterial inhibition (Transparence of the medium).

3. Results

3.1. Well diffusion method

Ethanolic extract of *Mirabilis jalapa* showed antibacterial activity against the tested organism when it used at concentrations: 500mg/ml, 250 mg/ml, 125 mg/ml and it were produced maximum zone of inhibition against *E. coli*(30, 20, 12 mm) and S. *aureus* (29, 18, 12 mm) respectively, Table 1 illustrated the results.

3.2. Disc diffusion method

Ethanolic extract of *Mirabilis jalapa* using disc diffusion method was displayed antibacterial activity against tested organism used at 500mg/ml, 250 mg/ml and 125 mg/ml, and it was produced maximum zone of inhibition against *E. coli* (20, 14.3, 8.3 mm) and for *S. aureus* it was: (24, 9, 7 mm), Table 2 illustrated the results.

3.3. Broth dilution method

No growth in the tube containing 50% of the ethanolic extract of *M. jalapa*, but there is a partial growth in tube containing 25% of the extract and obvious growth in tube containing 12.5% while an abundant growth observed in control tube.

Table 1: The mean diameter of inhibition zone (mm) using well diffusion method

Extract concentration	*S. aureus*	*E. coli*
50 mg/ml	29	30
25 mg/ml	18	20
12.5 mg/ml	12	12

Table 2: Mean diameter of inhibition zone (mm) using disc diffusion method

Extract concentration	*S. aureus*	*E. coli*
50 mg/ml	24	20
25 mg/ml	9	14.3
12.5 mg/ml	7	8.3

4. Discussion

The zone of inhibition of the ethanolic extract of *Mirabilis jalapa* seeds against *S. aureus* were found to be 29 mm, 18 mm and 12mm at 500 mg/ml, 250 mg/ml, 125 mg/ml respectively, using the well diffusion method. This result was in agree with the report of Subin Mary, et al, (2012)[14] who studied antibacterial activity of the leaf ethanolic extract of *Mirabilis jalapa* against *S. aureus*, and they found that the inhibition zone was 17 mm at 1mg/ml concentration.

Also it is in agree with Naveed, et al., (2010),[15] who reported that the ethanolic extract of only white flowered *Mirabilis jalapa* showed good antibacterial activity against *S. aureus.*

In this study the ethanolic extract of *Mirabilis jalapa* seeds when it was tested against *E. coli* by well diffusion method at the concentrations (500 mg/ml, 250 mg/ml and 125 mg/ml) was showed greatly inhibition zone as mean diameter of 30 mm, 20 mm and 12 mm respectively. This finding was in agreement with Poovenran et al, (2011),[16] who studied the ethanolic extract of *Mirabilis jalapa* leaf against different biofilm strain of *E. coli* and found the inhibition showing different zone of inhibition (22 mm – 24 mm) at 1000 mg/ml by well diffusion method.

The mean diameters using disc diffusion method of ethanolic extract of *Mirabilis jalapa* seeds at the three tested concentrations (500 mg/ ml, 250 mg/ml and 125 mg/ml) against *S. aureus* were found to be 24mm, 9 mm and 7 mm respectively. And against *E. coli* were: 20 mm, 14.3 and 8.3 mm respectivly. This result was agree with the report of Lakshmi et al (2010),[17] who found that methanolic extract of stem of *Mirabilis jalapa* were significantly effective against *S. aureus* in 20.66mm of inhibition zone and dichloromethane stem extract of *Mirabilis jalapa* showed significant effect against *E. coli* in 20.66 mm inhibition zone at 200 mg/ml when it was compare with other solvents.

Also it is in agree with Sumithra, et al. (2012)[18] who evaluated the antibacterial activity of various solvent extract of *Mirabilis jalapa* flowers in vitro against five organisms, *E. coli* and *S. aureus* using the disc diffusion method and found the inhibitory activity of ethanolic and methnolic extract of flowers was greatly effective with zone of (14 – 15mm).

The ethanolic extract of *Mirabilis jalapa*seeds was displayed significant effect against the tested organisms at the highest used concentration (500 mg/ml), and the least activity at the lowest concentration (125 mg/ml), that is mean the activity was found to be concentration dependent.

These activities were due to several phytoconstituent antimicrobial compounds such as flavonoids, tannins, saponins, polyphenols, alkaloids which are effective as antimicrobial substances against wide range of microorganisms.

5. Conclusion

The present work has been concluded that *Mirabilis jalapa* plant can be considered as potential drug for antibacterial action and it could be used as alternative treatment to orthodox antibiotic in the treatment of infectious diseases caused by different bacterial species.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Joseph I, Singh R. Antimicrobial Activity of Selected Medicinal Plants, Craetva magna (Linn.), Pongamia glabra (Linn.) and Areca catechu (Linn.). *Ethnobotany Leaflets*. 2008;12:995–1002.
2. Ramesh B, Nidavani, Mahalakshmi AM. An Ethno pharmacological Review of Four Oclok Flower Plant. *J Biol Sci Opin*. 2014;2(6):334–8.
3. Ramesh BN, Mahalakshmi AM, Mallappa SH. Towards abetter undrestanding of an Updated Ethno pharmacology of Celosia argentea L. *Int Jpharm Pharm Sci*. 2013;5(3):54–9.
4. Harsha VS. Antibacterial Activity of root of Lantana camera Linn. *Pharmacol Online*. 2011;2:1427–30.
5. Otimenyin OS, Mo U, Ogbonna A. Antimicrobial and hypoglycemic effects of Momordica balsamina. Linn. *J Nat Prod*. 2008;1:3–09.
6. Nitta T, Arai T, Takamatsu H, Inatomi Y, Murata H, Iinuma M, et al. Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin-Resistant Staphylococcus aureus. *J Health Sci*. 2002;48(3):273–6. doi:10.1248/jhs.48.273.
7. Britto AD, Gracelin DHS, Kumar P. Phytochemical studies on five medicinal ferns collected from Southern Western Ghats, Tamilnadu. *Asian Pac J Trop Biomed*. 2012;2:S536–8. doi:10.1016/s2221-1691(12)60268-8.
8. Peters HJ, Krumm AJ, Gonzales RR, Gunter KK, Paez KN, Zygowicz SD, et al. Multicultural Environments of Academic Versus Internship Training Programs: Lessons to Be Learned. *J Multicultural Counseling Dev*. 2011;39:114–24. doi:10.1002/j.2161-1912.2011.tb00145.x.
9. Eduega HO, Okwu DE, Mbuebie BO. Phytochemical constituents of some Nigerian Medicinal plants. *Afr J Biotechnol*. 2005;4(7):685–8.
10. Levin RA, Raguso RA, McDade LA. Fragrance chemistry and pollinator affinities in Nyctaginaceae. *Phytochem*. 2001;58:429–40. doi:10.1016/s0031-9422(01)00257-6.
11. Oladummoye MK. Comparative Evaluation of Antimicrobial Activities of leaf extract of Mirabilis jalapa. *Trends Med Res*. 2007;2(2):108–12.
12. Daniel M. Medicinal plants chemistry and properties. U.S.A: Science Publishers; 2006.
13. Sukhdev SH, Suman PSK, Gennaro L, Dev DR. United Nation Industrial Development Organization and the International Center for Science and High Technology. In: Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology; 2008. p. 116.
14. Mary S, Jayakar Z, Viswanad B, V, Gopal V, R. In vitro Anthelmintic activity of aerial parts of Mirabilis jalapa Linn. *International journal of pharmaceutical sciences review and research*. 2012;(12):107–110.
15. Antibacterial activity of genetic varients of Mirabilis jalapa. *Pharmacognacy J*. 2010;2(7):181–4.
16. Poovendran P. Antimicrobial activity of Mirabilis Jalapa and Dichrotachys cinerea against biofilm and extended spectrum of beta lactamase (ESBL) producing uropathogenic Escherichia coli. *Afr J Microbiol Res*. 2011;5(22):3620–3. doi:10.5897/ajmr11.116.
17. Lakshmi R, Nath, Manjunath KP, Savadi RV, Akki KS. Anti-inflammatory activity of Mirabilis jalapa Linn. leaves. *J Basic Clin Pharm*. 2010;001:93–6.
18. Sumithra P, Varalakshmi S, Devasena K. Phytochemical Analysis and Antibacterial Activity of Mirabilis jalapa Flower against Gastro Intestinal Pathogens. *Int J Sci Res*. 2014;3(12):1168–70.

Author biography

Abdulrahman M A Saeed, Associate Professor

Kawther A F Mahdi, Postgraduate Student

Cite this article: Saeed AMA, Mahdi KAF. Antibacterial effect of ethanolic extract of *Mirabilis jalapa* (Alsaa Arbaa) seeds against some bacteria. *Indian J Pharm Pharmacol* 2020;7(4):226-229.