

Analysis of antimicrobial activity of medicinal plant *Amaranthus viridis*

Sumaira Sarwar¹, Maimoona Sabir², S. Sajid Raza³, and S. A. Malik⁴

¹Assistant Professor, IMDC, Islamabad,
Islamabad Medical & Dental College, Islamabad, Pakistan

²Assistant Professor, Department of Microbiology,
University of Haripur, Pakistan

³Professor & Head of Department (Biochemistry),
Islamabad Medical & Dental College, Islamabad, Pakistan

⁴Professor, Department of Biochemistry,
Quaid-E-Azam University, Islamabad, Pakistan

Copyright © 2016 ISSR Journals. This is an open access article distributed under the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT: Present study was conducted to investigate the antimicrobial potential of *Amaranthus viridis* ethanolic extracts, against two Gram positive bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis*, and four Gram negative bacterial strains via; *Proteus vulgaris*, *Pseudomonas picketii*, *Klebsiella pneumonia* and *Escherichia coli*. Disc diffusion method was used and also by calculating their MIC values. Antibacterial assay indicated that *Amaranthus viridis* had inhibitory activity against *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas picketii*.

Agar tube dilution method was used to evaluate the antifungal activity of *Amaranthus viridis*. Ethanolic extract was tested against five different strains of fungal species, including; *Fusarium solani*, *Alternaria species*, *Aspergillus flavus green*, *Aspergillus nigar* and *Aspergillus fumigatus*. It was concluded that *Amaranthus viridis* had moderate antifungal activity (41-51%) against *Alternaria species* while low activity (below 40%) against *Aspergillus flavus green*, *Aspergillus nigar* and *Aspergillus fumigatus*. The study revealed antibacterial and antifungal potential of ethanolic extract of *Amaranthus viridis*.

KEYWORDS: Anti microbial activity, Medicinal plant, *Amaranthus viridis* ethanolic extract.

1 INTRODUCTION

The use of medicinal herbs in the treatment of infection is an age-old practice and several natural products are used as phytotherapeutic for treatment of many diseases. Human infections constitute a serious problem and most frequent pathogens are microorganisms such as bacteria and fungi [1].

From the very beginning, man is gifted with plants used for curing diseases or in preparation of new drugs. According to reports, 30% of the drugs are obtained from medicinal plants [2, 3]. Efforts have been made to detect the active constituents responsible for such properties in plants [4, 5]. However, the scientists showed an increasing interest to extract new antimicrobial bioactive compounds from various natural sources, like folk medicinal plants and extracts obtained from medicinal plants [3, 6, 7, 8].

According to World Health Organization [9], medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [10]. The search for discovery of new antimicrobial agents is necessary and stimulates the research of new chemotherapeutic agents in the medicinal plants [11,12].

Multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [13,14]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions [15]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [16], there is a constant need for new and effective therapeutic agents [17]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [18,19].

Leaves of *Amaranthus viridis* (30 g), gur (2g) and water Leaves are collected, boiled in water with 'gur' and filter through a cloth Half a cup twice daily before meal. Use for expulsion of worms, abdominal pain and diarrhea [21]. Leaves of *Amaranthus viridis* are used for treating eczema, psoriasis and rashes, constipation, inflammation, bronchitis, anemia and leprosy. It inhibits enzymes, plays regulatory role on different hormones and is used for anticancer, antihepatotoxic and protection of cardio vascular system [22].

The aim of the present investigation was to assess the antibacterial and antifungal activity of the crude ethanolic extracts of *Amaranthus viridis* and to compare the result to the effect of the antibiotics upon bacterial and fungal growth that could be useful for the development of new tools for the control of infectious diseases. The plant was chosen on the basis of its reported uses in literature.

2 MATERIALS AND METHODS

2.1 COLLECTION OF PLANTS

Plant material was collected from different areas of Rawalakot, District Poonch (AJK) Pakistan. The plant was identified as *Amranthus viridus*, by Plant Taxonomist, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

2.2 EXTRACTION

Fresh aerial plants were taken, rinsed with distilled water, kept under shade for drying at room temperature. The dried plants were then grinded to fine powder by simple maceration process. Total 100 gm of each powdered plant was taken and mixed in 250 L of ethanol; each plant mixture was kept for two weeks at room temperature. It was filtered using Whatman-41 filter paper. Then from filtrate ethanol was completely evaporated by rotary vacuum evaporator under reduced pressure. After evaporation 12 gm of *Amranthus viridus* was obtained. This extract was kept at 4 °C until use.

2.3 PREPARATION OF SAMPLES

250 mg of the extracts was dissolved in 10 ml of DMSO to get 25 mg/ml concentration. This stock solution was used for further dilutions with DMSO. Antibiotic disc of Amoxycillin was used as positive control, while Pure DMSO was used as negative control.

Two Gram positive bacterial strains; *Staphylococcus aureus* and *Bacillus subtilis* and four Gram negative strains; *Proteus vulgaris*, *Peudomonas picketij*, *Klebsiella pneumonia* and *Escherichia coli* were used for the assay.

2.4 PREPARATION OF BACTERIAL CULTURE

Nutrient broth medium (Merck) was prepared by dissolving 0.8 g/ml nutrient broth in distill water; pH, adjusted at 7.0. 10 ml of broth was dispensed in test tubes. Medium was autoclaved at 121°C for 20 minutes. Then single colony was inoculated from single colony culture plate of bacterial strain and incubated it at 37 °C for 24 hours.

2.5 AGAR DIFFUSION METHOD

In this method, after applying the samples and incubation at 37°C for 24 hours, the diameter of the clear zones around each disc was measured and compared against zones of inhibition around antibiotic discs.

2.6 ANTIFUNGAL ASSAY

The agar tube dilution method was used to evaluate the antifungal activity of *Amaranthus viridis*. Five fungal strains were used which were; *Fusarium solani*, *Alternaria species*, *Aspergillus flavus green*, *Aspergillus nigar* and *Aspergillus fumigatu*. The standard drug used was Terbinafine as positive control.

3 RESULTS

3.1 ANTIBACTERIAL ASSAY

Ethanol extract of *Amaranthus viridis* was tested against six different strains of bacteria. Two strains were gram positive, *Staphylococcus aureus* and *Bacillus subtilis*, whereas *Proteus vulgaris*, *Pseudomonas picketii*, *Klebsiella pneumoniae* and *Escherichia coli* were gram negative. All dilutions were made in DMSO which has no inhibitory effect on growth of bacteria. Five concentrations (max. 25 mg/mL and min. 5mg/mL) of this plants extract was tested against five bacterial strains. Zones of inhibition were measured finally by MIC test.

It was observed that after 48 hours of incubation the ethanolic extract of *Amaranthus viridis* leaves showed inhibitory activity against all the bacterial strains used except *Staphylococcus aureus*. Highest activity was seen against *bacillus subtilus* while moderate activity was shown against other strains used (Figure I)

3.2 ANTIFUNGAL ASSAY

Five different strains of fungal species, including; *Fusarium solani*, *Alternaria species*, *Aspergillus flavus green*, *Aspergillus nigar* and *Aspergillus fumigatus* were used. Result shows that *Amaranthus viridis* had moderate antifungal activity (41-51%) against *Alternari species* while low activity (below 40%) against *Aspergillus flavus green*, *Aspergillus nigar* and *Aspergillus fumigatus* (Figure II).

4 DISCUSSION

The Amaranthaceae family comprises many species with biological activities, which are used in nutrition and alternative medicine [23]. Screening programs for biologically active natural products require the right bioassays. They must be simple, inexpensive, rapid, and sensitive enough to detect active principles which are generally present only in small concentrations in crude extracts. Their selectivity should be such that the number of false positives is reasonably small. Antimicrobial assay is another rapid technique to discover new compounds that are effective against fungi and bacteria.

Present study indicates potent antibacterial activity of ethanolic extract of *Amaranthus viridis*. Most sensitive bacterial strains were Gram positive *Bacillus subtilis*, Gram negative *E.coli* and *Proteus vulgaris*. Antibacterial activity of *Amranthus viridis* ethanolic extract has been reported against *Bacillus subtilis* and *Escherichia coli* [23]. Present study results also agree with previously reported study [24].

Achyranthes bidentata Blume belonging to the family Amaranthaceae was investigated for antibacterial activity against *Bacillus subtilis*, *Pseudomonas pneumonia* and *Escherichia coli* [25], which is in accordance with our finding using ethanolic extract of *Amaranthus viridis* of the mentioned family against same bacterial strains.

Antimicrobial activity of *Alternanthera maritima* ethanolic extract of Amaranthaceae family against gram positive, gram negative bacteria and filamentous fungi were reported[26], which satisfies our finding that the extract used is active both against bacterial and fungal strains.

The study finding was in accordance with reported antibacterial and antifungal activity of *Amaranthus paniculatus* and *Achyranthes bidentata* seeds of Amaranthaceae family [27,28]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [20]. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use.

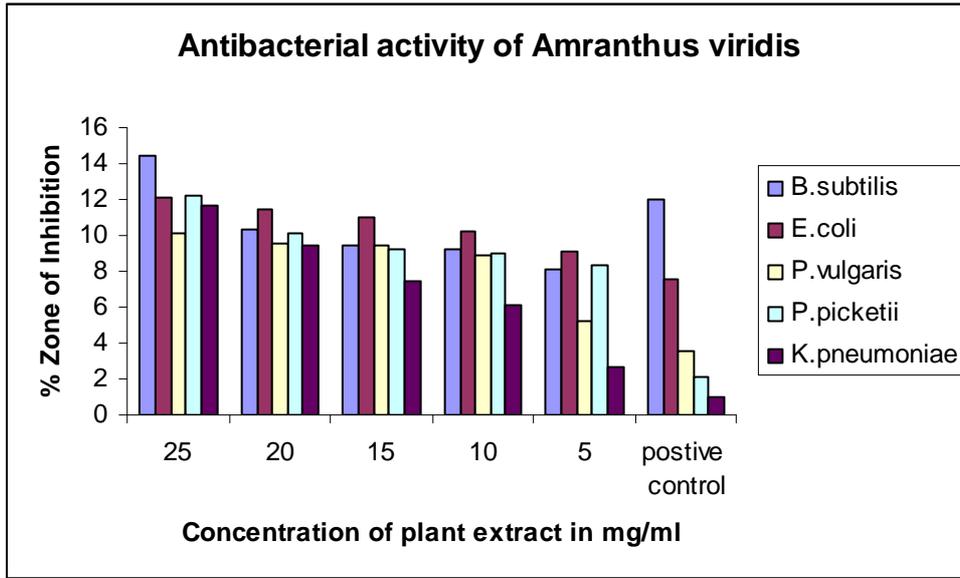


Fig1: Effect of different concentrations of the plant extract on different bacterial strains

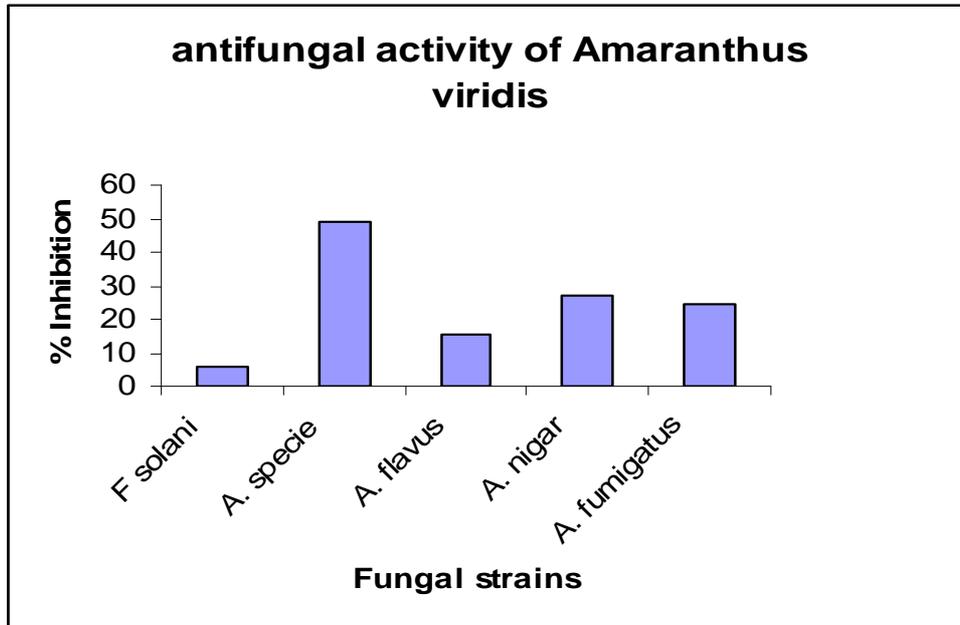


Fig 2: Effect of different concentrations of the plant extract on different fungal strains

Table 1: Antibacterial activity of ethanolic extracts of *Amaranthus viridis* against different bacterial strains

Bacterial strains	Mean diameter of Zones of inhibition (mm) \pm S.E							
	Conc. 25 mg/mL	Conc. 20 mg/mL	Conc. 15 mg/mL	Conc. 10 mg/mL	Conc. 5 mg/mL	DMSO	Amoxicillin	Gentamicin
<i>Bacillus subtilis</i>	13.1 \pm 0.01	11.3 \pm 0.05	9.5 \pm 0.05	9.3 \pm 0.06	8.1 \pm 0.03	0.00 \pm 0.00	12.2 \pm 0.3	36.1 \pm 0.61
<i>Escherichia coli</i>	12.4 \pm 0.08	11.5 \pm 0.03	10.2 \pm 0.05	10.1 \pm 0.03	9.6 \pm 0.06	0.00 \pm 0.00	17.2 \pm 0.05	26.1 \pm 0.11
<i>Proteus vulgaris</i>	10.1 \pm 0.06	9.65 \pm 0.03	9.45 \pm 0.05	8.95 \pm 0.06	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.08	26.1 \pm 0.61
<i>Pseudomonas pickettii</i>	12.5 \pm 0.06	0.1 \pm 0.06	9.8 \pm 0.03	9.3 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	14.8 \pm 0.03	23.1 \pm 0.61
<i>klebsiella pneumoniae</i>	11.1 \pm 0.06	7.3 \pm 0.01	6.1 \pm 0.03	2.3 \pm 0.06	6.0 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	9.05 \pm 0.03
<i>Staphylococcus aureus.</i>	0.0 \pm 0.00	0.00 \pm 0.00	0.0 \pm 0.00	0.00 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	0.00 \pm 0.00	0.0 \pm 0.00

The data represents the mean \pm SEM value of three replicates

Table 2: Antifungal activity of ethanolic extract of *Amaranthus viridis*

S.No	Name of fungus	Linear Mean Growth in -ive control(mm)	Linear Mean Growth with extract(mm)	Mean inhibition % \pm S.E	Standard Drug	Morphological changes
1	<i>Fusarium solani</i>	100 \pm 0.00	100 \pm 0.00	0.00 \pm 0.00	Turbinafine	Spore formation inhibition
2	<i>Alternaria species</i>	49.5 \pm 0.06	25.2 \pm 0.00	49.09 \pm 0.06	Turbinafine	-
3	<i>Aspergillus flavus g</i>	100 \pm 0.00	85.0 \pm 0.01	15.2 \pm 0.01	Turbinafine	-
4	<i>Aspergillus nigar</i>	100 \pm 0.00	72.5 \pm 0.03	27.4 \pm 0.03	Turbinafine	-
5	<i>Aspergillus fumigatus</i>	100 \pm 0.00	75.1 \pm 0.08	24.8 \pm 0.01	Turbinafine	-

The data represents the mean \pm SEM value of three replicates

5 CONCLUSION

The results revealed that ethanolic extracts of *Amaranthus viridis* is effective against tested bacterial and fungal strains. These plants could be a source of new antibiotic compounds, so further studies are required, including in vitro and in vivo investigations, toxicity evaluation as well as purification of active antibacterial and antifungal constituents.

ACKNOWLEDGMENTS

This study was supported by grants from Quaid-i-azam University, Islamabad. Authors would like to acknowledge Dr Waseem Shahzad and Yasarib Arafat who contributed to this research work, we also acknowledge cooperation of laboratory staff, Department of Biochemistry, Quaid-i-azam University Islamabad, Pakistan.

DECLARATION OF INTEREST

The authors have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- [1] Wheat, L. J. *Fungal infection in the immunocompromised host*, in: Rubin, R. H and Young, L. S, (Eds.), Clinical approach to infection in the compromised host, Vol.3. New York: Plenum Publishing Corporation, pp. 211-237, 1994.
- [2] S. Grabley and R. Thiericke, "Bioactive agents from natural sources:trends in discovery and application", *Adv Biochem Eng Biotechnol*, vol. 64, pp. 101-54, 1999.
- [3] M. R Khan, W. Rizvi, G. N Khan, R. A Khan, S. Sheen, Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata*. *J Ethnopharmacol*, vol. 122, pp. 91-99, 2009.

- [4] Hostettmann, K. Marston, A. Wolfender, J. L., *Strategy in the search for new biologically active plant constituents*, in: Hostettmann K, Marston A, Maillard M, Hamburger M, editors. *Phytochemistry of Plants Used in Traditional Medicine*, Proceedings of the Phytochemical Society of Europe. Oxford, Oxford Science Publications, pp. 18-45, 1995.
- [5] F.L Ansari, S. Nazir, H. Nourine, B. Mirza, "Combinatorial synthesis and antibacterial evaluation of an indexed chalcone library", *Chem Biodivers*. Vol. 2, pp. 1656–1664, 2005.
- [6] M. Hanif, M. Hussain, S. Ali, M.H Bhatti, M. S Ahmed, B. Mirza, H.S Evans, "Synthesis, spectroscopic investigation, crystal structure and biological screening, including antitumor activity, of organotin (IV) derivatives of piperonylic acid", *Turk J Chem*, vol. 31, pp. 349–361, 2007.
- [7] John J. Gavin, "Analytical microbiology, II. The diffusion methods", vol. 5, pp. 25-33, 1956.
- [8] J. N. Ellof, "Which extractant should be used for the screening and isolation of antimicrobial components from plants", *J Ethnopharmacol*, vol. 60, pp. 1-6, 1998.
- [9] G. Bisignano, A. Tomaino, R. L. O Cascio, G. Crisafi, N. Uccella, A. Saija, "On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol", *J Pharm Pharmacol*, vol. 51, pp. 971-974, 1999.
- [10] J. C. Siqueira and E. F. Guimarães, "Amaranthaceae do Rio de Janeiro – gênero *Alternanthera* Forskal", *Rodriguésia*, vol. 36, pp. 21-40, 1984.
- [11] J. Davis, "Inactivation of the antibiotics and the dissemination of resistance genes," *Science*, vol. 264, pp. 375-382, 1994.
- [12] R. F. Service, "Antibiotics that resist resistance," *Science*, vol. 270, pp. 724-727, 1995.
- [13] I. Ahmad, Z. Mehmood, F. Mohammad, "Screening of some Indian medicinal plants for their antimicrobial properties", *J Ethnopharmacol*. Vol. 62, pp. 183-193, 1998.
- [14] S. Monroe and R. Polk, "Antimicrobial use and bacterial resistance", *Curr Opin Microbiol*. vol. 3, pp. 496-50, 2000.
- [15] S. M. Bhavnani and C.H. Ballow, "New agents for Gram-positive bacteria", *Curr Opin Microbiol*. vol. 3, pp. 528-534, 2000.
- [16] A. M. Clark, "Natural products as resource for new drugs", *Pharm Res*, vol. 13, pp. 1133-114, 1996.
- [17] G.A. Cordell, "Biodiversity and drug discovery a symbiotic relationship" *Phytochemistry*, vol. 55, pp. 463-480, 2000.
- [18] O.T. Erdogru, "Antibacterial activities of some plant extracts used in folk medicine" *Pharmaceutical Biol*. vol. 40, pp. 269–273, 2002.
- [19] J. Samin, A.K. Mir, D. Siraj, M. Waheed, H. Manzoor, G. Aneela G, "Herbal remedies used for gastrointestinal disorders in Kaghan valley, NWFP, Pakistan", *Pak J Weed Sci Res*, Vol. 14, pp. 169-200, 2008.
- [20] K.B.S. Ashok, K. Lakshman, K.N. Jayaveera, N.V. Krishna, M. Manjunath, M.V. Suresh, "Estimation of rutin and quercetin in *Amaranthus viridis* L. by HPLC", *Asian Journal of Experimental Science*, vol. 23(1), pp. 51-54, 2009.
- [21] S. Voravuthikunchai, A. Lortheeranuwat, W. Jeeju, T. Sririrak, S. Phongpaichit, T. Supawita, "Effective medicinal plants against Enterohaemorrhagic *Escherichia coli* O157: H7", *J Ethnopharmacol*; vol. 94, pp. 49–54, 2004.
- [22] D. Veeramuthu, A. Muniappan, I. Savarimuthu, "Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. India BMC Complement," *Altern Med*, vol. 6, pp 35-38, 2006.
- [23] B. R. Balakrishnan, B. Sangameswaran, B. Arul B, V. H. Bhaskar, "Antibacterial activity of aerial parts of *Achyranthes bidentata* Blume", *Indian J Pharmaceutical Sci*, vol. 65(2), pp. 186-188, 2003;
- [24] M. J. Salvador, P. S. Perira, S.C. Franca, R. C. Candido, I. Y. Ito, D. A. Dias, "Comparative study of Antimicrobial and Antifungal activity of calus culture and adult plants extracts from *Alternanthera maritima* (Amaranthaceae)", *Brazilian J Microbiology*, vol. 34, pp. 131-136, 2003.
- [25] D. Janovska, K. Kubikova, L. Kokoska, "Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine", *Czech J Food Science*, vol. 21 (3), pp. 107-110, 2003.