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Article

Antibacterial and Antioxidant Activities of Slender Amaranth Weed

Atividades Antibacteriana e Antioxidante de Caruru-de-Mancha

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ABSTRACT - In the present study, antibacterial and antioxidant [1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity] activities of a weed, slender amaranth (*Amaranthus viridis* L.) were investigated. Extracts of different plant parts were prepared in *n*-hexane, chloroform and ethyl acetate. Antibacterial activity was measured by using 100 mg mL^{-1} concentration extracts against 4 deadly phytopathogenic bacterial species viz. *Pseudomonas syringae* Van Hall, *Ralstonia solanacearum* Smith, *Erwinia carotovora* (Jones), Holland and *Xanthomonas axonopodis* Hasse. In antioxidants assays, 10, 20 and 30 mg mL^{-1} extracts were used keeping DPPH as control. In these bioassays, ethyl acetate fraction of *A. viridis* leaf exhibited the best antibacterial and antioxidants activity. Ethyl acetate leaf fraction showed the highest inhibition zone diameter (IZD) where it caused 21 mm IZD against *P. syringae* and 19 mm IZD against *E. carotovora*. This extract also showed 22, 52 and 84% antioxidant activity at 10, 20 and 30 mg mL^{-1} concentrations, respectively. Previously there is no report available that describes antibacterial activity of root extract of *A. viridis* against *P. syringae*. Moreover, antioxidant activity of stem and root extracts in *n*-hexane, chloroform and ethyl acetate was investigated first time in the world. It was concluded that the biological activities observed during the present investigation may be due to the presence of bioactive constituents that can be harnessed as natural antibacterials and antioxidants.

Keywords: *Amaranthus viridis*, bioactive components, DPPH, phytopathogens, weeds.

*RESUMO - Nesta pesquisa foram estudadas possíveis atividades antibacterianas e antioxidantes da planta daninha caruru-de-mancha (**Amaranthus viridis** L.), com o uso da solução DPPH (1,1-diphenyl-2-picrylhydrazyl, para remoção da atividade de radicais livres). Extratos de diferentes partes dessa planta foram preparados em n-hexano, clorofórmio e acetato de etila. A atividade antibacteriana foi medida utilizando extratos de concentração de 100 mg mL^{-1} contra quatro espécies bacterianas fitopatogênicas mortais: **Pseudomonas syringae** Van Hall, **Ralstonia solanacearum** Smith, **Erwinia carotovora** (Jones) Holland e **Xanthomonas axonopodis** Hasse. Nos ensaios com antioxidantes foram utilizados extratos de 10, 20 e 30 mg mL^{-1}, mantendo o DPPH como controle. Nesses bioensaios, a fração acetato de etila da folha do caruru-de-mancha apresentou a melhor atividade antibacteriana e antioxidante. A fração foliar do acetato de etila apresentou o maior diâmetro da zona de inibição (IZD), onde causou 21 mm de IZD contra **P. syringae** e 19 mm de IZD contra E. carotovora. Esse extrato também apresentou 22%, 52% e 84% de atividade antioxidante nas concentrações de 10, 20 e 30 mg mL^{-1}, respectivamente. Não existem na literatura relatos disponíveis que descrevam a atividade antibacteriana do extrato de raiz de **A. viridis** contra **P. syringae**. Além*

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disso, a atividade antioxidante dos extratos de raiz e caule em n-hexano, clorofórmio e acetato de etila foi investigada pela primeira vez no mundo. Concluiu-se que as atividades biológicas observadas durante a presente investigação podem ser devidas à presença de constituintes bioativos, os quais poderiam ser aproveitados como antibacterianos naturais e antioxidantes.

Palavras-chave: *Amaranthus viridis*, componentes bioativos, solução DPPH, fitopatógenos, plantas daninhas.

INTRODUCTION

Plant pathogens cause serious threat and are main reason for crop losses (Wang and Jin, 2017). Crop infection due to bacterial pathogens leads to the low yield of food. Innovative disease prevention techniques are now adopted in crops, to avoid crop impairments during development, harvest and postharvest treatments, as well as to attain maximum yield and to assure agricultural sustainability (Fang and Ramasamy, 2015).

Plants are confronted with a number of serious bacterial pathogens. e.g., pear and apple are infected by *Erwinia amylovora,* which causes bacterial disease, fire blight (Mikiciński et al., 2016). Causal agent of bacterial canker of kiwifruit is *Pseudomonas syringae* (Cimmino et al., 2017). Bacterial leaf spot produced in lettuce is induced by *Xanthomonas campestris* (Fayette et al., 2016). Solanaceous plants are affected by bacterial wilt, caused by *Ralstonia solanacearum* (Kim et al., 2016). *Xanthomonas axonopodis* causes bacterial blight of onion (Robène et al., 2015) and Cassava bacterial stem rot is caused by *Erwinia carotovora* (McCallum, 2017).

In agriculture, synthetic chemicals such as pesticides are being used for pest control. However, excessive use of these chemicals has resulted in food contamination, negative environmental impacts and antimicrobial resistance, which together have caused drastic impacts on food security and human health (Sribanditmongkol et al., 2012; Wu et al., 2014; Singh and Trivedi, 2017). Therefore, there is dire need to use nature friendly methods for pest control (Kim et al., 2017).

At present, search for novel use of natural products linked to pest management for plants is very active (Droby et al., 2009). One strategy to control plant pathogens is to explore natural bioactive components from weeds. These components have antimicrobial and radical scavenging properties (Dhankhar et al., 2013). Reactive oxidative species (ROS) in plants are surrounded by inevitable discharge of electrons onto O_2 from the electron transport activities in the cell (Heyno et al., 2011). At high range, reactive oxidative species are detrimental to plants, animals and human beings. When range of reactive oxidative species exceeds its optimal level then the cell is considered to be in the state of oxidative stress. When the cell is under environmental stress, the assembly of ROS increases that depicts that the cell is at risk by triggering oxidation of proteins, enzyme inhibition, peroxidation of lipids, initiation of programmed cell death pathway and at last leading to death of the cells (Maheshwari and Dubey, 2009).

There are few reports regarding better antibacterial and antioxidant activities of genus Amaranthus. The betacyanin fraction from *Amaranthus dubius* demonstrated a better antimicrobial activity than that of *Hylocereus polyrhizus* against nine Gram-positive bacterial strains. Similarly, the *A. dubius* fraction was more active than the *H. polyrhizus* fraction against five Gram-negative bacterial strains (Yong et al., 2017). Leaf extracts of *A. viridis* along with other weeds namely *Oxalis corniculata*, *Chenopodium album*, *Solanum nigrum* and *Convolvulus arvensis* were reported to have antimicrobial potential against phytopathogenic bacteria viz. *Xanthomonas campestris*, *P. syringae*, *Morganella morganii*, *Acinetobacter baumannii* and *Xylophilus* sp. (Akhtar et al., 2016). Iqbal et al. (2012) evaluated the antioxidant activity of leaf and seed extract of *A. viridis* in pure and aqueous methanol by using DPPH free radical scavenging test and reported IC_{50} (14.25–83.43 µg mL^{1}) against leaf and 46.50–75.91 µg mL^{-1} against seed extract, respectively.

As natural products are getting attention worldwide because these are inexpensive, safer and environment friendly (Martínez et al., 2017), therefore, weeds can be used for the management of bacterial pathogens. Although there are number of reports regarding antibacterial and antioxidant activities of *A. viridis* around the globe but such studies are scarce on plant species

growing wild in Pakistan, especially against plant pathogenic bacteria. Therefore, the present investigation was designed to explore antibacterial and antioxidant activities of *Amaranthus viridis* L. against bacterial plant pathogens as well as their antioxidant activity that can be useful in tackling plant bacterial diseases as well as their use as antioxidants.

MATERIALS AND METHODS

Plant material

Amaranthus viridis was collected from wild places, district Sialkot, Pakistan. Sialkot lies 256 meters above sea level and located at 32.4945° N, 74.5229° E. Different plant parts viz. leaf, seed, shoot and root were separated from *A. viridis* and sun dried for 7 days. These dried plant parts were fine powdered and stored at 4 °C till further use.

Extraction from plant material

One hundred grams each of powdered leaf, seed, stem and root were soaked in methanol (500 mL) and incubated for 7 days at 25 °C. After this incubation, the plant materials were filtered through Whatman filter paper No. 1. The filtrate thus obtained was then concentrated at 45 °C under vacum, using rotary evaporator (Model: Laborata 4000/Gl, Heildoph Germany). This process was repeated three times to obtain methanolic extracts of all plant parts. Extra solvent from the methanolic filtered extracts was evaporated to obtain methanol free extract. This methanol free extract was reconstituted in distilled water (dH_2O) and partitioned using 3 organic solvents viz. *n*-hexane, chloroform and ethyl acetate, in order of their increasing polarity. The resulting fractionated extracts were evaporated under vacuum as in case of methanolic extract to get solvent free fractionated extracts. This procedure was done in order to fractionate secondary metabolites contained in the methanolic extracts of *A. viridis* leaf, seed, shoot and root (Akbar et al., 2017).

Bacterial cultures

Bacterial cultures were procured from Culture Bank of Pakistan (CBP), University of the Punjab, Lahore, Pakistan. These cultures were; *Pseudomonas syringae* (Accession No. 0405), *Ralstonia solanacearum* (Accession No. 0407), *Erwinia carotovora* (Accession No. 0421) and *Xanthomonas axonopodis* (Accession No. 001). All bacterial cultures were maintained in the laboratory in Lysogeny Broth (LB) medium slants/Petridishes and stored at 4 °C till further use.

Antibacterial assays

Antibacterial activity of different organic extracts viz. *n*-hexane, chloroform and ethyl acetate extracts of leaf, seed, stem and root was determined using disc diffusion method. 100 mg each of *n*-hexane, chloroform and ethyl acetate extracts of leaf, seed, stem and root was dissolved in 200 µL of Dimethyl sulphoxide (DMSO) and final volume was raised to 1 mL by adding autoclaved dH_2O to make 100 mg mL^{-1} concentration solution of all extracts. All bacterial species were grown on LB medium in Petriplates. Six mm diameter filter paper discs were cut with the help of paper punch and then these discs were sterilized. These discs were placed onto the streaked bacterial cultures contained in glass Petriplates. Fifty micro liters of 100 mg mL^{-1} solution of *n*-hexane, chloroform and ethyl acetate extracts of leaf, seed, stem and root of *A. viridis* were loaded onto each disc. So each disc contained 5 mg of respective extract. Control treatments received 50 µL of 200 µL mL^{-1} DMSO. These Petriplates were incubated at 37 °C in an incubator. After 72 hours, antibacterial activity in terms of inhibition zone (IZ) was measured with the help of measuring scale.

Determination of antioxidant activity (DPPH radical scavenging assay)

For the determination of antioxidant activity of test weed, concentrated leaf, seed, stem and root of *A. viridis* extracts in *n*-hexane, chloroform and ethyl acetate were used as described by

Rashid et al. (2017). All partitioned extracts were utilized for assessing DPPH radical scavenging activities. All these extracts were dissolved in methanol (99.9%) at 10, 20 and 30 µg mL^{-1} and final volumes were raised up to 4.0 mL. After that, 1 mL of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was poured into these solutions and shacked the solution for 1 minute. These solutions were kept in dark for 15 minutes and after that absorbance were recorded at 517 nm by using spectrophotometer (Model: UV3000 Spectrophotometer). Control solution in cuvettes received DPPH solution while treatments received DPPH solution + solution of respective extracts. All treatments used in these experiments were made in triplicate and their mean values were taken to determine DPPH radical scavenging activity by using the following formula.

$$\text{Inhibition (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

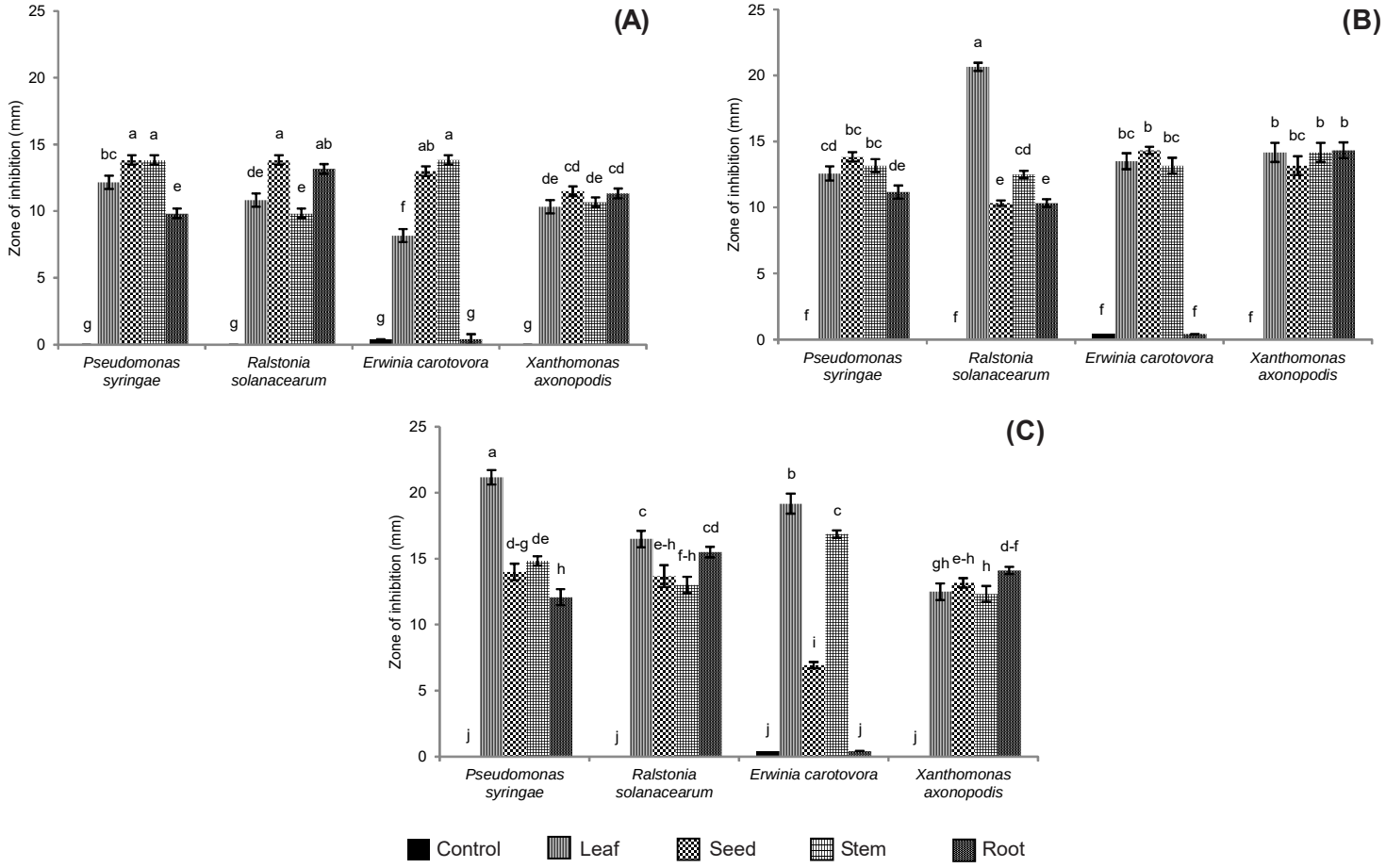
Statistical analysis

For statistical analysis, ANOVA was done followed by Fisher's LSD test using Minitab Statistical Software- Minitab 17.

RESULTS AND DISCUSSION

Antibacterial activity of organic solvent extracts

Data regarding antibacterial activity of leaf, root, seed and stem extracts of *A. viridis* in *n*-hexane, chloroform and ethyl acetate are shown in (Figure 1). Leaf, seed, stem and root extracts in *n*-hexane showed inhibitory effects against all bacterial species while DMSO that was kept as control did not show any inhibitory effects except in case of *E. carotovora*. In case of *n*-hexane, leaf extract showed 12.16, 10.83, 8.17 and 10.33 mm inhibition zone diameter (IZD), whereas seed extract exhibited 13.83, 13.83, 13 and 11.5 mm IZD, stem showed 13.83, 9.83, 13.83 and 10.67 mm IZD, while root depicted 9.83, 13.17, 0.41 and 11.33 mm IZD against *P. syringae*, *R. solanacearum*, *E. carotovora* and *X. axonopodis,* respectively. On the other hand, chloroform extract of *A. viridis* depicted 12.58, 20.66, 13.5 and 14.17 mm IZD in case of leaf, while seed extracts showed 13.83, 10.33, 14.33 and 13.17 mm IZD whereas, stem extract showed 13.18, 12.5, 13.17 and 14.17 mm inhibition, while, root extract revealed 11.17, 10.33, 0.41 and 14.33 mm IZD against *P. syringae*, *R. solanacearum*, *E. carotovora* and *X. axonopodis,* respectively. Ethyl acetate extract *A. viridis* exhibited pronounced bactericidal activity against all test bacteria. Ethyl acetate extract *A. viridis* leaf showed 21.17, 16.5, 19.17 and 12.5 mm inhibition zone while seed revealed 14, 13.67, 6.93 and 13.17 mm IZD whereas, stem extract marked 14.83, 13, 16.87 and 12.33 mm IZD and root showed 12.08, 15.5, 0.43 and 14.11 mm inhibition zone diameter against *P. syringae*, *R. solanacearum*, *E. carotovora* and *X. axonopodis,* respectively. DMSO (control) also exhibited non significant inhibitory effects (0.41-0.43 mm IZD) against *E. carotovora* and similar effects were also observed in treatments and this effect was non significant when compared with control. So it became evident that none of the *A. viridis* root extracts exhibited any significant inhibitory effects against *E. carotovora.* These results reveal the higher potency of ethyl acetate extract of *A. viridis* leaf against the test microorganisms. The highest inhibitory effects were observed in case of leaf extract of *A. viridis* against *P. syringae* and *E. carotovora* where 21.17 and 19.17 IZD was recorded, respectively. Root extract in all solvents was also found ineffective against *E. carotovora* and slight inhibition zone recorded in this bacterial species may be attributed to the effect of DMSO as is evident in control also. Previously Malik et al. (2016) reported antimicrobial activity of methanol and chloroform extract of leaves and stem of *A. viridis* against *Pseudomonas* spp. by using disc diffusion method where 15 and 13 mm inhibition zones were recorded, respectively. In another investigation, *Oxalis corniculata* and *A. viridis* leaf extracts in water, methanol and ethanol exhibited antibacterial activity and caused 42, 40 and 44 mm inhibition zone against *X. campestris* and *P. syringae*, respectively (Akhtar et al., 2016) Similarly, Willcox et al. (2014) reported that seed extract of *A. viridis* showed 2 mm inhibition against *Ralstonia.* In another study, *Amaranthus* extract of leaf stem and root in ethyl acetate showed 62% inhibitory activity against *X. axonopodis* (Xia et al., 2016). Similarly, Mushtaq et al. (2012) reported 15 mm, 22 and 32 mm inhibition against *P. syringae, R. solanacearum* and



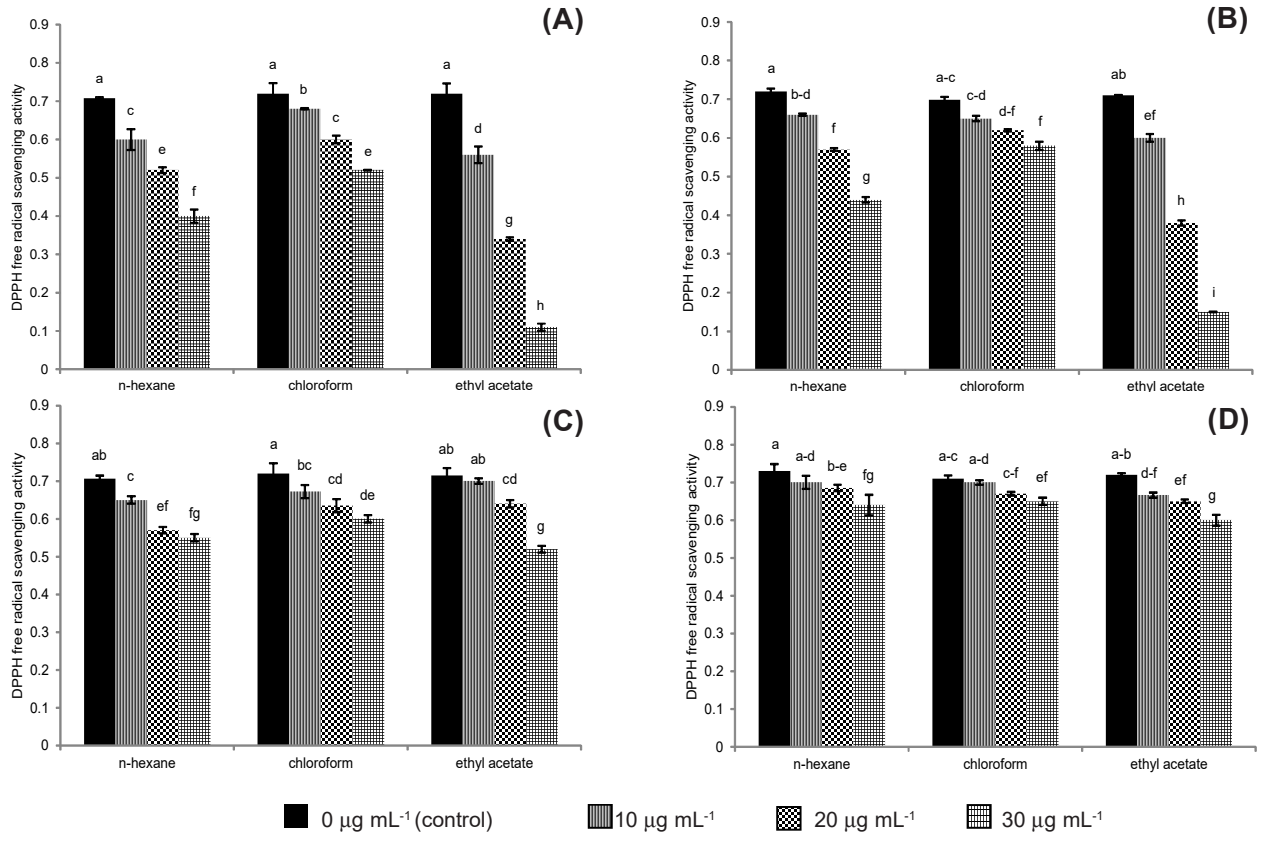
Values with different letters at their top show a significant difference (P≤0.05), as determined by Fisher's LSD test. Vertical bars show standard errors of means of three replicates.

Figure 1 - Effect of different concentrations of (A) n-hexane, (B) chloroform and (C) ethyl acetate extracts leaf, seed, stem and root of *Amaranthus viridis* on growth of *Pseudomonas syringae*, *Ralstonia solanacearum*, *Erwinia carotovora* and *Xanthomonas axonopodis*.

X. axonopodis, respectively, by using methanolic extract of leaf and stem of *A. viridis*. Previously there were no investigations reported on root extract of *A. viridis* against *P. syringae* anywhere in the world.

Antioxidant activity of organic solvent extracts

Data regarding antioxidant activity of *A. viridis* are shown in (Figures 2 and 3). *n*-hexane extract of leaf exhibited (15, 26 and 43%), Seed (8.3, 20.8 and 38.9%), stem (8, 19.4 and 22.2%) and root (4.1, 6.2 and 12.3%) at 10, 20 and 30 µg mL^{-1} concentrations, respectively. However, chloroform fraction of leaf showed 5, 16 and 27%, while seed showed 7, 11.3 and 17% stem showed 6.7, 11.8 and 16.7% whereas, root marked 1.4, 5.6 and 8.4% antioxidant activity at 10, 20 and 30 µg mL^{-1} concentrations, respectively. On the other hand, ethyl acetate fraction of leaf showed 22, 52, 84%, while seed showed 15.5, 46.5 and 78.9% stem showed 2, 10.5 and 27% whereas, root marked 7.5, 9.7 and 16.7% antioxidant activity at 10, 20 and 30 µg mL^{-1} concentrations, respectively. These results show better antioxidant activity of leaf followed by seed extracts over stem and root of *A. viridis*. So these parts can be exploited for the extraction of antioxidant compounds for multiple purposes. On the contrary, stem and root exhibited less antioxidant activity, which may be attributed to less quantity of antioxidant compounds contained in these two parts of plant. In case of leaf and seed, antioxidant activity of these two plant parts in all organic solvents tested was found significant at all concentrations tested, while in case of stem and root, not all concentrations were found active as in case of ethyl acetate extract of



Values with different letters at their top show a significant difference (P≤0.05), as determined by Fisher's LSD test. Vertical bars show standard errors of means of three replicates.

Figure 2 - Antioxidant (DPPH free radical scavenging) activity of *n*-hexane, chloroform and ethyl acetate extract of (A) leaf, (B) seed, (C) stem and (D) root of *Amaranthus viridis*.

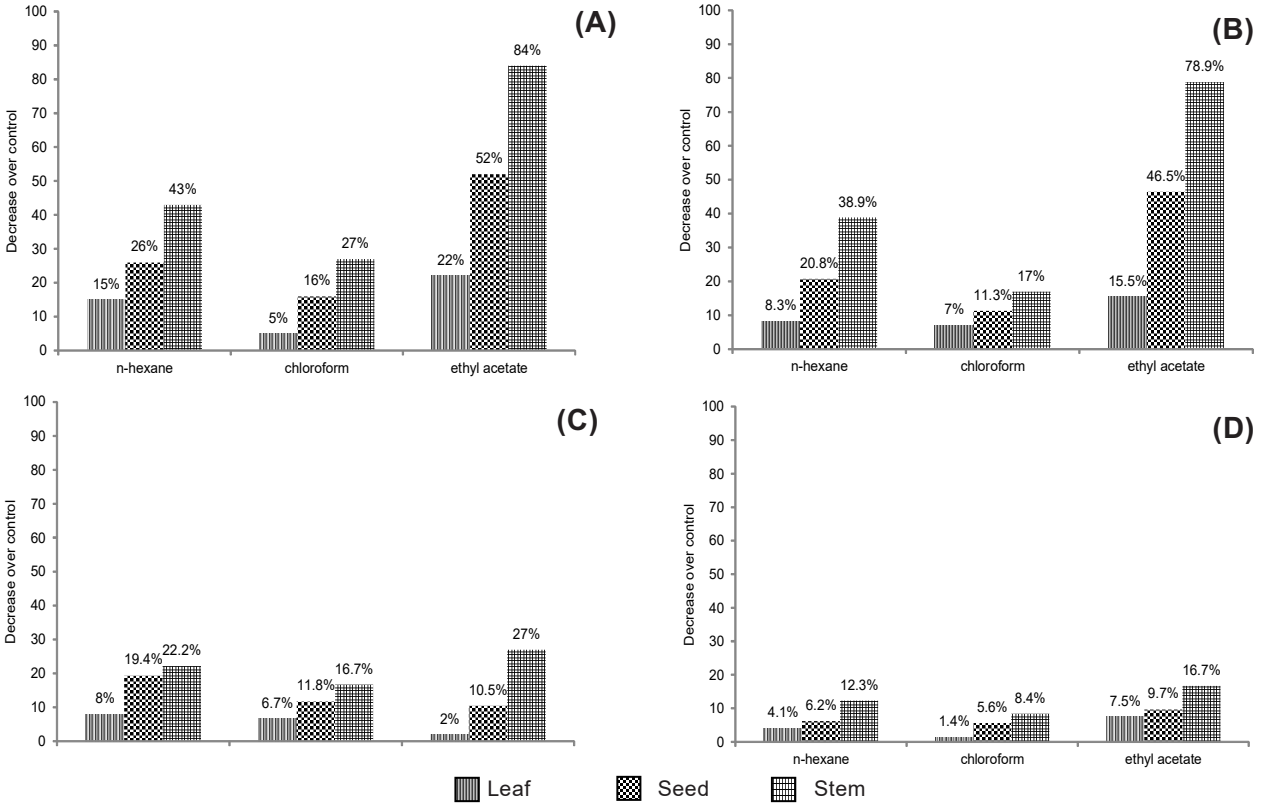


Figure 3 - Percentage reduction in DPPH free radical scavenging activity (A) leaf, (B) seed, (C) stem and (D) root of *Amaranthus viridis* due to different concentrations of *n*-hexane, chloroform and ethyl acetate extract over control.

stem as well as chloroform and ethyl acetate extract of root at 10 µg mL^{-1} concentration (Figure 3). Similar results were reported by Iqbal et al. (2012), where methanolic extract of leaf and seed of *A. viridis* exhibited good 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity as revealed by IC_{50} (14.25 - 83.43 µg mL^{-1}). Malik et al. (2016) also reported DPPH free radical scavenging activity of *A. viridis* in methanolic extract upto 76%. In the present research work, antioxidant activity of stem and root extracts in *n*-hexane, chloroform and ethyl acetate was investigated first time around the world.

The present research work concludes that amongst extracts of different parts of *A. viridis*, leaf exhibited best antibacterial and antioxidant activity. Ethyl acetate extract of leaf incurred 21 mm IZD against *P. syringae*. Similarly, this leaf extract in ethyl acetate showed 84% antioxidant activity in terms of DPPH radical scavenging activity. This shows the presence of potent antibacterial as well as antioxidant moities that should be explored further in order to isolate and identify bioactive compounds that can be used as new potent antibacterials as well as antioxidants.

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