

Comparison of Phytochemical and Antimicrobial activities of *Amaranthus cruentus* (Red Amaranthus) grown in Hydroponic system and in traditional soil system

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Abstract- Hydroponics has emerged as one of the most popular agricultural production methods today. However, whether hydroponically produced plants are of comparable quality to that of soil grown plants is still unclear and a lot of research is going on this matter. This study is also a part of research on phytochemical, and antimicrobial activity of ethanol and aqueous extract of plant *Amaranthus cruentus* grown in hydroponic system and traditional soil system. *Amaranthus cruentus* is naturally gluten-free and a good source of calcium, zinc, copper, vitamin B6, folate, and an excellent source of fiber, iron, magnesium, phosphorus, and manganese. Growth data revealed that it appears morphologically far better in hydroponic system as compared to that of soil system. In a hydroponic setup, dry weight was increased by seven times. Flavonoid and Mineral elements content is found to be higher in hydroponic systems. The diameter of inhibition zone of *Amaranthus cruentus* grown in hydroponic system is higher as compared to that of soil system. While, Minimum inhibitory concentration (MIC) is less in case of hydroponic system as compare to soil system. This study concludes that the hydroponic system for growing of plants is good choice.

Keywords: Hydroponics, Soil grown plants and Nutrients,

Introduction

A wide variety of indigenous and minor crops has been utilized for daily consumption since ancient times. They are not only important ingredients of unique gastronomic dishes but also traditional functional food to maintain wellness. In order to elucidate such a phenomenon as well as to seek highly effective plants, a number of plant extracts and isolated compounds have been tested for their bioactivity by various *in-vitro* model systems. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in tropical countries. Furthermore, it is expected that the wide use and extension in the utilization of such local agricultural products would increase and stabilize the income of farmers in the rural areas.

Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic

drugs to which many infectious microorganisms have become resistant. Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two-fold namely they provide key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine to be used for the treatment of disease.

Amaranthus cruentus Linn. collectively known as amaranth or pigweed is an annual flowering plant in the family Amaranthaceae that yield staple amaranth grain⁽¹⁻⁴⁾. It is a tall annual herb with clusters of dark pink flowers. The plant can grow up to 2m in height, and blooms in summer to fall (Flora of Tamil Nadu, VOL. II, 1987). The present study was undertaken to assess antimicrobial and phytochemical property of *Amaranthus cruentus*.

Amaranthus cruentus commonly used as a leaf vegetable. Some time they are grown as ornamentals, for fodder, and for making dye. Dried plant is burnt for the preparation of potash. It is given to lactating mothers for treating constipation, anemia, kidney complaints. Roots are boiled with honey and given to infants for their laxative effect, its aqueous extract is used to treat pains in the limbs, as a tape worm expellant and wound dressing and tumors. It has antioxidant properties.

Material and Methods

Hydroponic set up

Amaranthus cruentus was grown using the ebb and flow method in a hydroponic system. The technique made use of PVC pipes with 3-inch diameter pores. A steady flow of nutritional solutions was maintained in the growth channels. The plants were planted in net pots with cocopeat and lacca balls as the support

medium, and the roots were hung in a nutritious solution running through the channels. The reservoir's feeding solution was pumped out. The flow was directed into the other channel, which was equipped with an end cap and spout at the decreasing end of the increasing channel. Before returning to the reservoir, the nutrient solution passed through all of the system's channels (closed system). To prevent contamination of the nutrient solution, a reservoir with a surface area of 0.053 m² was placed beneath the growing system and covered⁽¹⁰⁻¹³⁾.

The *Amaranthus cruentus* plant was chosen as the source material for this objective. This experiment was divided into two groups, one was carried out in the soil of HWC field, and the other was in the laboratory of Himalaya Wellness Company, Faridabad. *Amaranthus cruentus* seeds were purchased from a local nursery and verified by Dr. M.R. Uniyal, Ex. Advisor Medicinal Plant, U.P., Govt. Three replicates of 20 seeds were placed in a tray filled with cocopeat and moistened with water. Similarly, seeds were grown in a greenhouse in the soil as well. At 3, 4 leaves stage three replicates of 10 good plants were transferred in a hydroponic setup and exposed to an external environment. In the hydroponic setup, we have used modified Hoagland solution as a nutrient media with pH 6.9. 10 L nutrient media were added every week and maintained the EC with nutrient media and pH with ortho-phosphoric acid and lastly, phytochemical and antimicrobial activity analysis was done.

Preparation of plant extract

The crude plant extract was prepared using the Soxhlet extraction technique. About 20 g of powdered plants material was evenly packed into a thimble and extracted with 250 ml of solvents. As a solvent, acetone was used. The extraction procedure is repeated for another 24

hours or until the extractor's syphon tubes solvent becomes colourless. The extracts was then placed in a beaker and cooked on a hot plate at 30°C-40°C until the solvent had evaporated completely. The dried extract was kept at 4°C in the fridge for future study.

Phytochemical Analysis

Quantification of total phenolic compounds-

The Folin-Ciocalteu reagent technique was slightly modified to detect the quantity of phenol in the aqueous extract. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 2% Na₂CO₃ solution were added to 1 ml of plant extract. The resultant mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765 nm. Gallic acid (1 mg/ml) was utilized as a standard. All of the tests were performed triplicate. The findings were computed and represented as gallic acid equivalent (mg/g of extracted substance) using the standard curve.

Quantification of flavonoid- To determine flavonoid content, the aluminium chloride colorimetric method was used with some modifications. 1 ml of the sample plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water for 30 minutes at room temperature. At 420 nm, the absorbance was measured. As a standard, 1 mg/ml of quercetin was used. All of the tests were carried out in triplicate. The flavonoid content was calculated using the standard curve and expressed as quercetin equivalent mg/g of extracted compound.

Anti-Microbial Activity

Strains of tested organisms

The bacterial strains used in the study were obtained from the Microbiology Laboratory, Department of Quality control and Quality assurance, Himalaya Wellness Company,

Faridabad, Haryana. Two Gram positive bacteria that is *S. saprophyticus*, *E. faecalis* and four Gram negative bacteria that is *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Proteus vulgaris* were used for this study.

Antibacterial activity

The antibacterial efficacy of both methanolic leaf extracts of *Amaranthus cruentus* was tested by agar well diffusion method. The collected clinical isolates were grown in Muller Hinton broth (Himedia, Mumbai, India) at 37°C for 24 hrs. with constant agitation in a shaker. The cultures from broth were aseptically swabbed on sterile Muller Hinton agar (Himedia, Mumbai) plates using sterile cotton swabs. The wells of 6 mm were punched in the inoculated plates using a sterile borer. Aliquots of 100 µl of methanolic leaf extracts (50 mg/ml in dimethyl sulphoxide) were transferred into labelled wells. The wells were also filled with 50 µl positive (Amikacin, 10 mg/ml in dimethyl sulphoxide) and 50 µl negative (dimethyl sulphoxide only) controls. The plates were incubated at 37°C for 24 h in upright position and the zones of inhibition were recorded. The activity assays were conducted in triplicate.

Determination of MIC by Microlitre plate assay

The microtitre plate was prepared in aseptic conditions. A stock solution of test sample (10% w/v) was prepared in dimethyl sulphoxide. A volume of 100 µl of test material was filled in first row of the plate. To all other wells 50 µl of sterile nutrient broth was filled. The test material was transferred to the next well to attain serial dilutions. To each well, 30 µl of resazurin indicator solution (0.02%) was added. Finally 10 µl of bacterial suspension (1x10⁸ CFU/ml) was added to each well. The plate was set with positive control and a column with all solutions except

test compound and negative control; a column contains 10 µl of sterile nutrient broth except test compound and bacterial suspension. The plates were prepared and incubated at 37°C for 24 h. The colour change from purple to pink indicated a positive response. The lowest concentration at which colour change was noted was the minimum inhibitory concentration (MIC) values for the test material and bacterial strain⁽¹³⁻¹⁶⁾.

Results and Discussion

Our data have clearly shown that flavonoids and total phenol are more in hydroponically grown plants (Figure-1), whereas in soil cultivated plants, it is less. The phenolic compounds are among the most numerous and widespread groups of plant metabolites. They have biological properties such as anti-apoptosis, antiaging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, endothelial function improvement, and inhibition of angiogenesis and cell proliferation. Natural antioxidants are primarily found in plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols, and so on. Flavonoids are hydroxylated phenolic substances that plants produce in response to microbial infection and have been shown to be antimicrobial *in-vitro* against a wide range of microorganisms. In the case of phenolic compounds, soil cultivated plants possess more. Polyphenols, saponins, tannins, and oxalates are phytochemicals found in *amaranth* grain that are not considered nutrients but may be antinutrient factors. Cooking reduces these compounds' content and anti-nutrient effect.

The results of inhibitory effect of methanolic leaf extracts of *Amaranthus cruentus*. hydroponically grow and soil grow are shown

in Table-1. The results show that different bacterial species exhibit different sensitivities towards the extract. The extract was found to be inhibitory to all bacterial isolates but with variable extent. The order of activity against selected bacteria was *E. coli* > *P. vulgaris* > *P. aeruginosa* > *K. pneumoniae* > *S. saprophyticus* > *E. faecalis* and. Different plant metabolites have shown effective antibacterial activity against uropathogens including drug resistant strains. In the present study, the *A. cruentus* (L) leaf methanolic extract effectively inhibited all bacteria tested. The zone inhibition values of the extract against tested bacteria ranged from 14.2±0.55 to 18.3±0.55 mm in hydroponically grow methanolic extract while soil grow show range from 13.3±0.55 to 17.5±0.55mm. Amikacin showed inhibition zones that ranged from 15.8±0.57 to 23.2±0.57 mm. The both methanolic extracts exhibited maximum activity against *E. coli* (hydroponic 18.3±0.55 mm and soil grow 17.5±0.55) followed by *P. vulgaris* (16.5±0.57 mm and 16.0±0.57) and then against *P. aeruginosa* (15.6±0.57 mm and 15.3±0.57 mm). The results in the present study indicate that the antibacterial activity varies according to type of bacteria used for the study. The least activity was exhibited by *E. faecalis* with the smallest zone (14.2±1.15 mm and 13.8±1.15 mm) and inhibited at lowest concentration 1.25 (mg/ml) (Table 2). The antibacterial activity of tested *A. cruentus* was compared with the standard drug amikacin. The cell growth was evaluated using resazurin, an oxidation-reduction indicator. A change in colour from blue to pink indicated the growth of bacteria, and the minimal inhibitory concentration (MIC) was noted as lowest concentration of the test compound that prevented this change in colour. The MIC of methanolic leaf extract ranged from 5.5 to 0.37 mg/ml. The results

from MIC indicated that *E. coli* was the most sensitive microbe to the *A. cruentus* leaf extract grown in hydroponic and soil system

being negatively affected at lowest concentration tested 0.37 (mg/ml) and 0.37 (mg/ml) respectively.

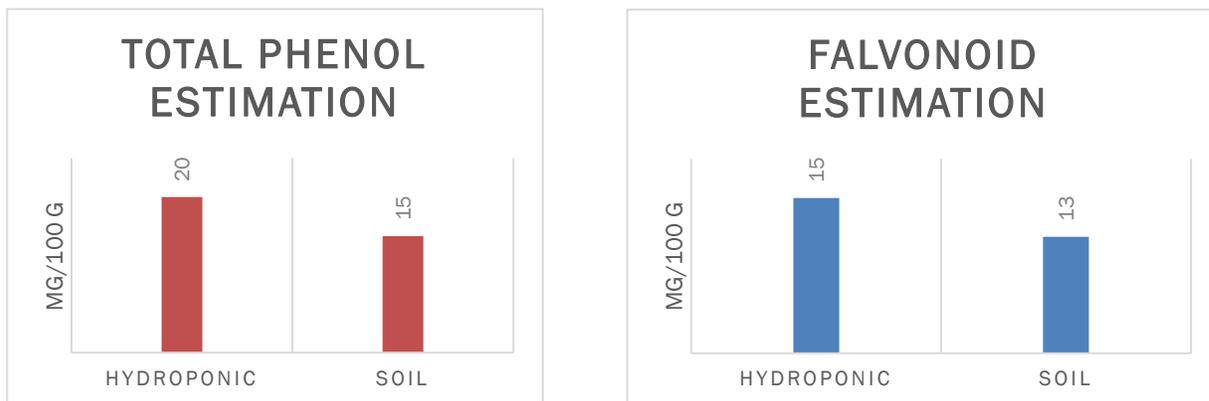


Figure-1 Comparison of flavonoids and phenolic compounds in *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

Table-1 Comparison of antimicrobial activity of *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

S. No.	Microorganism	Zone of Inhibition Hydroponically grown (mm)	Zone of Inhibition soil grown (mm)	Zone of inhibition of Amikacin* (mm)
1	<i>S. saprophyticus</i>	15.4±0.57	14.3±0.57	20.2±0.57
2	<i>E. faecalis</i>	14.2±1.15	13.8±1.15	15.8±0.57
3	<i>E. coli</i>	18.3±0.57	17.5±0.57	23.2±0.57
4	<i>P. aeruginosa</i>	15.6±0.57	15.3±0.57	17.5±0.57
5	<i>K. pneumoniae</i>	14.9±1.15	14.5±1.15	19.2±0.57
6	<i>P. vulgaris</i>	16.5±0.57	16.0±0.57	20.3±1.15

*Standard antibiotic

Table-2 Minimum inhibitory concentration (MIC) *Amaranthus cruentus* plant extracts grown in soil and hydroponic system.

S. No.	Microorganism	MIC mg/ml Hydroponically grown	MIC mg/ml soil grown
1	<i>S. saprophyticus</i>	5.0	5.5
2	<i>E. faecalis</i>	2.3	2.5
3	<i>E. coli</i>	0.37	0.38
4	<i>P. aeruginosa</i>	1.24	1.26
5	<i>K. pneumoniae</i>	0.60	0.62
6	<i>P. vulgaris</i>	0.60	0.62

Conclusion

The comparative study of *Amaranthus cruentus* conclude that every domain like phytochemical and antibacterial studies in hydroponic as well as in soil reveals that hydroponics technology is better for

harvesting of *this* plant not only because of improved quality, but also because of higher yield, system ease of operation, and water efficiency. Small-scale farmers should use an open field hydroponic system instead of a controlled environment hydroponic system

because it requires less capital. Further research into hydroponic production and optimization of its ability to assure acceptable product quality and the selection of suitable types to deliver better hydroponic products is also worthwhile.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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