



Evaluation of Antioxidant Activity of Amaranth Species

**Rajput Chitra,* Gaikwad Vaishnavi ,Kamble Akansha ,Khade Pratiksha ,
Pawar Poonam Suryawanshi Pranali, Yadav Anisha**

Adarsh College of Pharmacy Vita

Abstract: The plant of *Amaranthus* species is utilized for treatment of some common diseases. Moreover the plant contains various biological active constituent such as Flavonoids, Sterol, Glycoside amino acid lipid phenolic acid and other micronutrients. Therefore, it is necessary to exploit it to its maximum potential in the medicinal and pharmaceutical fields. *Amaranthus cruentus*, is a plant species with a long history of traditional medical applications. Phenolic compounds in plants are of considerable increasing interest and becoming a subject of research due to their bioactive properties like antioxidant, antimicrobial etc. The object of this research was to determine antioxidant activity of Pet ether extract of *Amaranthus cruentus*, Radical scavenging activity done by DPPH method 100 ug/ml Ascorbic acid shows 18.22% radical scavenging activity while 100ug/ml *Amaranthus cruentus* Pet ether extract shows 16.71% radical scavenging activity.

Key words: Antioxidant activity, Ascorbic acid, DPPH method.

Introduction:

Amaranthus information

Amaranth, also known as *amaranthus*, is one of the oldest plant crops and has a great tolerance for drought, salinity, alkalinity, and acidic soil conditions. It is a member of the *Amaranthaceae* family, which has 850 species and 65 genera. *Amaranthus* is a genus of 50–60 species that are grown for their leaves. *Amaranthus cruentus*, also known as *A. caudatus*, *A. hybridus*, *A. hypochondriacus*, *A. bilitum*, *A. tricolor*, *A. gangeticus*, *A. tristis*, *A. melonch*, *A. managostanus*, and *A. polygamus*, is one of the main *Amaranthus* species.^[1] *Amaranthus* is a crop with rapid growth that is primarily grown in Latin America, Africa, and Asia. *Amaranthine*, a component of the broad class of substances known as *betacyanins*, is found in *amaranth*.^[2]



Fig. no.1. *Amaranthus cruentus*

Amaranthus cruentus

Amaranth is presently an underutilized crop despite its high content of micronutrients/bioactive phytochemicals and its capacity to thrive in harsh environmental condition. The present study aimed at determining the health benefits of *Amaranthus cruentus* L. in terms of protection against DNA damage induced by the mycotoxin aflatoxin B1 (AFB1) and oxidative stress using comet assay. ^[3]

Therapeutic uses

Amaranthus leaves have good nutritional value, few antinutrition factors, and a large amount of bioactive compounds. These substances may possess several health benefits, such as antioxidant, antimicrobial, antifungal, antihyperglycemic, and antihypercholesterolemic effects. The consumption of many grains has been associated with a lower risk of degenerative diseases that depend on oxidative stress, namely atherosclerosis, cancer, diabetes, alzheimer's, constipation, gastrointestinal disease, heart attack, chronic cardiavascular disease. Perceived deficiencies of essential vitamins (vitamins B1, B2, B3, B6, and B9) and minerals present a significant restriction on human health and economic growth. ^[4]

Pharmacological profile

1. Anti-nutritional activity
2. Anti-hyperglycemic activity
3. Anti-hypercholesterolemic activity
4. Anti-degenerative activity
5. Anti -protein coagulation activity
6. Anti-lipid peroxidation activity
7. Anti-diabetic activity
8. Anti-alzheimer activity
9. Anti atherosclerosis activity
10. Antifungal activity
11. Antimicrobial activity
12. Antioxidant activity.
13. Anti inflammation activity
14. Anticancer activity
15. Anti hypertention activity
16. Antibacterial activity
17. Anti parkinson activity

Scientific classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Caryophyllales
Family	Amaranthaceae
Genus	Amaranthus
Species	Amaranthus cruentus L.

Therapeutic uses

Amaranth has medical benefits such as decreasing cholesterol, antioxidant, anticancer, an tiallergic, and antihypertensive activities due to its high protein level and amino acid com position^[4]Antiamnestic, antithrombotic, immunomodulating, opioid, regulating, antioxidant, ligand, activating ubiquitin-mediated proteolysis, immunostimulating, embryotoxic, protease inhibiting, and antihypertensive due to its active peptides were found in amaranth Proteins. ^[1] This suggests that amaranth lunasin is a more effective peptide for preventing cancer. A lipid-transfer protein contains the amaranth lunasin peptide, and not in conjunction with a Bowman-Birk protease inhibitor, as a soybean was reported. Plants that contain lunasin can bolster fresh study on amaranth as a substitute food, contains peptides with health-promoting properties. ^[6]

Extraction

The initial stage in separating the desired natural products from the raw ingredients is extraction. According to the extraction principle, there are several different extraction procedures, including solvent extraction, distillation, pressing, and sublimation. The most common technique is solvent extraction. ^[7] Some of the popular extraction techniques includes,

1. Maceration
2. Digestion
3. Decotion
4. Infusion
5. Percolation
6. Microwave extraction
7. Soxhlet extraction.

One of the most commonly used methods for removing analytes from solid materials is soxhlet extraction after being discovered in 1879. In this technique, a finely powdered material was put in the thimble chamber of the Soxhlet apparatus (also known as a hot continuous extraction). Heat from the bottom flask causes the extraction solvent to vaporise into the sample vial, condense in the condenser, and drip back. The process is continued once the liquid is once again dumped into the bottom flask when it reaches the syphon arm. ^[8]

Extraction

Plant matter can be either fresh (like a leaf of a plant) or dry. To create more surface area, it needs to be crushed using a pestle and mortar. In our studies, we used an average of 14 g of *Amaranthus cruentus* powder in a 25 x 80 mm thimble, so there should be enough plant material to fill the porous cellulose thimble round bottom flask with 250 ml of ethanol added as the solvent is connected to a Soxhlet extractor and condenser mounted on an isomantle. The Soxhlet extractor's thimble is filled with the crushed plant material after being placed inside. Glass wool is used to lagged the side arm. The solvent is heated by the isomantle and starts to evaporate as it passes through the device and reaches the condenser. Once within the reservoir holding the thimble, the condensate drips. When the solvent level reaches the syphon, it pours back into the flask, restarting the cycle. It should take the procedure 16 hours to complete.

Following the completion of the procedure, the ethanol should be evaporated using a rotary evaporator, leaving a tiny yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. ^[9]

Antioxidant

Definition of Antioxidants

Antioxidant is also known as the “against oxidation”. An oxidant is a molecule Capable of inhibiting the oxidation of other molecule. As a preservative antioxidant plays important role in monitoring health of human being & also preserving the quality of the food. ^[13]

An antioxidant or a free radical scavenger, is a molecule Capable of decreasing or preventing the oxidation of free radical. ^[10]

Source of antioxidants

Vitamin E, α -carotene, lycopene, selenium, polyphenol, vitamin C

The three main antioxidant sources are glutathione, peroxidase, and cysteine.

There are significant levels of antioxidants such polyphenols, vitamin C, and vitamin E in fruit juices, beverages, and hot liquids.

According to epidemiological studies, the best defense against the onset of diseases like cancer, coronary heart disease, obesity, type 2 diabetes, hypertension, and cataract that are put on by oxidative stress is provided by fruits, vegetables, and less processed staple foods.

Functions of antioxidant

Antioxidants are only dietary supplements that the Food and Drug Administration (FDA) classifies as being taken in addition to regular food consumption in an effort to keep from certain diseases. It has been proven that eating fruits and vegetables frequently lowers the risk of developing chronic illnesses. An antioxidant-rich diet has a highly good long-term health benefit, according to studies. John proposed four potential methods in 1989 for how antioxidants might slow down the oxidation of fats and oils. These include adding lipid to the antioxidants, donating electrons, donating hydrogen, and making a complex of the lipid and the antioxidants. ^[11]

DPPH2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is the most commonly used antioxidant assay for plant extract.

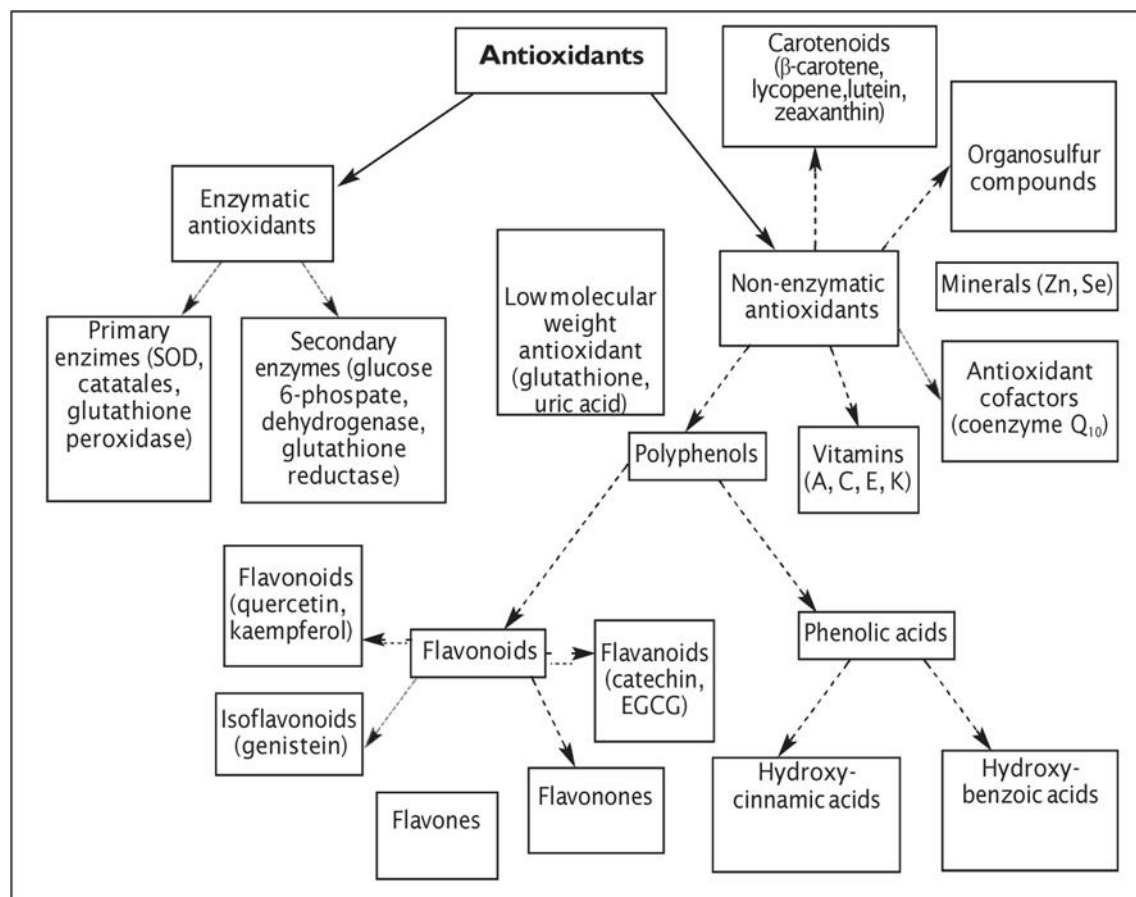
Principle of DPPH activity

This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to formation of the non-radical Form of DPPH. Advantages of DPPH Method

The application of this test allows the comprehension of the various chemical phenomena and has obvious advantages, like low cost, ease of performing experiments, reproducibility, applicability at room temperature, as well as automation possibilities.

Classification: (48-FTIRAOAC)

FITR Spectrophotometric methods used For Antioxidant Activity Assay in Medicinal plant.



Trending Marketed drug for antioxidants-

Health kart HK vidats Glutatathion

Heathkark HK Nitole vitamin E with evening primrose oil.

True Bacis Glutathione + Nutroxsun.

Himalayan Organics plant Based Nitamin E

Materials and Methods

Collection of plant materials

The leaves of *Amaranthus* species *Amaranthus cruentus* were collected from a farm in Suryanagar, Vita Tal, Khanapur Dist. Sangli, Maharashtra, India, in February 2023 and authenticated by the Museum of Botany, Balwant College, Vita, Dist: Sangli.

Antioxidant activity

1. Chemicals

Sr. No.	Chemicals	Company/Make
1	Methanol	Research lab fine chem. Industries, India
2	DPPH	Sigma
3	Ascorbic acid	Research lab fine chem. Industries, India

2. Instruments

Sr. No.	Instrument	Make/Model
1	Heating mantel	Rolex
2	UV	Shimatzu 1800

3. Experimental models

Soxhlet apparatus: for extraction

Experimental Work

Preparation of crude extract

The collected leaves of *Amaranthus cruentus* were shade dried under normal condition. Then the powder is ground into uniform powder using mixer. The 10g leaf powder of *Amaranthus cruentus* was extracted using Soxhlet apparatus for 6 to 8 hours with the methanol solvent. Extracts were filtered, evaporated and weighted.

Determination of Antioxidant activity

1. Antioxidant activity in the sample *Amaranthus cruentus* compounds was estimated for their free radical scavenging activity by using DPPH (1, 1 Diphenyl-2, Picryl- Hydrazyl) free radicals.
2. 100µL of *Amaranthus cruentus* (1mg/ml) were taken in the micro titer plate.
3. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition.
4. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm.
5. Radical scavenging activity was calculated by the following equation. ^[12]

DPPH radical scavenging activity (%) =

$$[(\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control})] \times 100$$

Results**1. Antioxidant activity**

Sr. No.	Compounds	Abs	Mean	Percentage of DPPH radical scavenging
1	Control	2.001	2.595	11.79%
		3.021		
		2.764		
2	Std. ascorbic acid	2.121	2.122	18.22%
		2.223		
		2.022		
3	<i>Amaranthus cruentus</i>	2.642	2.625	16.71
		2.636		
		2.601		

Table no. 1. Antioxidant activity of *Amaranthus cruentus* against DPPH**Discussion****Antioxidant Activity**

Amaranthus cruentus have rich source of phytoconstituents. *Amaranthus cruentus* show very good activity with 16.71 % DPPH radical scavenging activity. The standard ascorbic acid shows DPPH radical scavenging activity.

Conclusion**Antioxidant activity**

Antioxidants are becoming ever more interesting to scientists in the food field and medical professionals due to their protective roles in food products against oxidative deterioration and in the body against oxidative stress-mediated pathological processes. *Amaranthus cruentus* have high amount of phenolic compounds to show good antioxidant activity.

References:

1. Mahomoodally MF, Gurib-Fakim A, Subratty AH. Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmaceutical Biology*. 2005 Jan 1;43(3):237
2. Arendt EK, Zannini E. *Cereal grains for the food and beverage industries*. Elsevier; 2013 Apr 9.
3. Sarker U, Oba S. Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species. *Scientific reports*. 2019 Dec 31;9(1):20413
4. Wolosik K, Markowska A. *Amaranthus Cruentus* taxonomy, botanical description, and review of its seed chemical composition. *Natural Product Communications*. 2019 May;14(5):1934578X19844141.
5. Silva-Sánchez C, De La Rosa AB, León-Galván MF, de Lumen BO, de León- Rodríguez A, De Mejía EG. Bioactive peptides in amaranth (*Amaranthus hypochondriacus*) seed. *Journal of agricultural and food chemistry*. 2008 Feb 27;56(4):1233-40.
6. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese medicine*. 2018 Dec;13:1-26.
7. Bitwell C, Sen IS, Luke C, Kakoma MK. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*. 2023 Feb 11:e01585.
8. Nostro A, Germano MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in applied microbiology*. 2000 May 1;30(5):379-84.
9. Kislik V. Competitive Complexation/Solvation Theory of Solvent Extraction. I. General Theory. *Science Direct Working Paper*. 2002 Jul(S1574-0331):04.[9]
10. Moharram HA, Youssef MM. Methods for determining the antioxidant activity: a review. *Alexandria Journal of Food Science and Technology*. 2014;11(1):31-42.[10]
11. Yadav A, Kumari R, Yadav A, Mishra JP, Srivastava S, Prabha S. Antioxidants and its functions in human body-A Review. *Res. Environ. Life Sci*. 2016 Nov;9(11):1328-31.
12. Akin-Idowu PE, Ademoyegun OT, Olagunju YO, Aduloju AO, Adebo UG. Phytochemical content and antioxidant activity of five grain amaranth species. *Am. J. Food Sci. Technol*. 2017 Nov 14;5(6):249
13. Sehwal S, Das M. Antioxidant activity: an overview. *Int. J. Food Sci. Technol*. 2013;2:10