



## Comparative Estimation of Phenol and Flavonoid Content of Medicinally Important Plant – *Amaranthus carentus*

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**Abstract :** *Amaranthus cruentus* is a medicinal plant commonly found as a leafy vegetable. It belongs to family Amaranthaceae and distributed all over the world. *A. cruentus* has high nutritional value. Protein content of Amaranth grain is much higher than other grains like wheat and rye. Amaranth seeds, seed oil and leaves are used for health benefits such as to reduce blood pressure, cholesterol and weight, increase immunity, treat anemia, gastro intestinal tract disorders, antioxidant properties and anti inflammatory properties. Lunasin, a peptide in Amaranth seeds is considered to exert anti cancer properties. The consumption of *A. cruentus* products is advised for patients with celiac disease, therefore for diabetic persons. Owing to these properties, the present study was designed to investigate the total phenol and flavonoid content of various extracts of *A. cruentus* aerial parts spectrophotometrically. Extracts were prepared using solvents of different polarity ranging from semi polar to polar.

The total phenol contents in the extract was calculated as pyrocatechol equivalent ( $r^2 = 0.934$ ). It ranges from 341.47 to 1611.66 mg/g. Phenol contents in ethyl acetate extract are significantly lower whereas highest in ethanol extract. The total flavonoid content in the extract was calculated as quercetin equivalent ( $r^2 = 0.993$ ). Total flavonoid ranges from 1.44 to 4.95 mg/g. Flavonoid quantities in aqueous extract are significantly lower whereas highest in ethanol extract. This preliminary study is certainly useful for further biological study. These results provide data that make it promising to classify extracts in respect to their antioxidant potential.

**Keywords :** *Amaranthus carentus*, Amaranthaceae, Spectrophotometer, Phenol and Flavonoid.

### Introduction

The medicinal plants have been in the focus as life saving drugs right from beginning of human civilization. The medicinal plants have been the object of research in both classical and advanced areas of plant sciences. Natural products have regained its importance in recent years and several laboratories all over the world are engaged in isolation, purification and identification of bio- active principles from natural source. Plants are able to synthesize a multitude of organic molecules/ phytochemical referred to as “secondary metabolites”<sup>1, 2</sup>. Medicinal plants are rich source of secondary metabolites, exerting specific physiological effect on mammalian system and hence called active principles.

These molecules play variety of role in the life span of plants, ranging from structural ones to protection. Phenol compounds are regarded as one such group that is synthesized by plants during development<sup>1-3</sup> and in response to conditions such as infection, wounding, UV radiation<sup>4,5</sup> etc. Phenols are associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components and allelopathy<sup>6-8</sup>. Compounds of phenol show an array of health promoting benefits in human health.

Flavonoids, one of the important groups of secondary metabolites, are water soluble phenolic glycosides. Their contribution to physiological functions such as seed maturation and dormancy has already been established<sup>9</sup>. A simple definition describes flavonoids as “any group of substances found in fruits and vegetables essential for processing vitamin- C and needed to maintain capillary wall”. They may aid in protecting against infection. Deficiency can result in bruise. These flavonoids display a remarkable array of biochemical and pharmacological actions *viz.*, anti inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anti carcinogenic activities<sup>10</sup>. These compounds appear to play vital roles in defence against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy<sup>11</sup>. Quercetin, a type of flavanol, works as anti inflammatory, antioxidant, anticancer agents<sup>12</sup>.

*Amaranthus* species are of great importance in American people's diets<sup>13, 14</sup> particularly *A. cruentus* and *A. hybridus* have a high nutritional value<sup>15-21</sup>. The consumption of *A. cruentus* products is advised for patients with celiac disease and, therefore, also for diabetic persons<sup>19</sup>. *A. hybridus* has been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrization properties<sup>22</sup>; the products are used particularly for stomach aches, diarrhoea, and dysentery. *A. cruentus* and *A. hybridus* leaves are used as a vegetable<sup>23</sup> and sauces prepared from these plants are recommended for convalescent patients<sup>24</sup>. These two species are reputed to promote health and a long shelf life.

In spite of the abundance use in the traditional and ethno medicinal systems, scientific studies to explore the pharmacological studies of these plants have not been carried out yet. The present work is carried out in order to estimate the comparative efficacy of the plant in view of phenol and flavonoid content.

## Experimental

UV-Vis S1700 Pharma spectrophotometer (Schimadzu) was used for the measurement of absorbance. All solvents used were of AR-grade and were obtained from Merck, Mumbai (India).

### Collection of Plant Materials

Fresh plant material was collected from Pune, Maharashtra, India. The plant material was taxonomically identified and authenticated by the Botanical survey of India, Pune. Its authentication number is BSI/ WRC/ Cert/2015/AV02.

### Extraction

Air shade dried and powdered plant material (10 g) was extracted with solvents (50ml) of different polarity by keeping it for 24 hours at room temperature. Solvent was recovered under reduced pressure to obtain crude extract.

### Determination of Total Phenols

The total phenol contents of aerial parts of plant material were determined using Folin-Ciocalteu reagent and sodium carbonate according to the method described by Malik and Singh<sup>25</sup>. The concentration of phenol was determined as equivalent of phenol /g of extract by measuring absorption at 650 nm using pre-calibrated standard curve employing pyrocatechol. Experiment was carried out in triplicate and results were recorded as mean  $\pm$  SEM

### Determination of Total Flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts<sup>26</sup>. Each extract of the plant material in methanol was mixed with Aluminium chloride and

sodium-potassium tartarate. It was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The concentration of flavonoid in the test extracts was calculated from the calibration plot and expressed as mg quercetin equivalent /g of extract. Experiment was carried out in triplicate and results were recorded as mean  $\pm$  SEM.

**Results and Discussion**

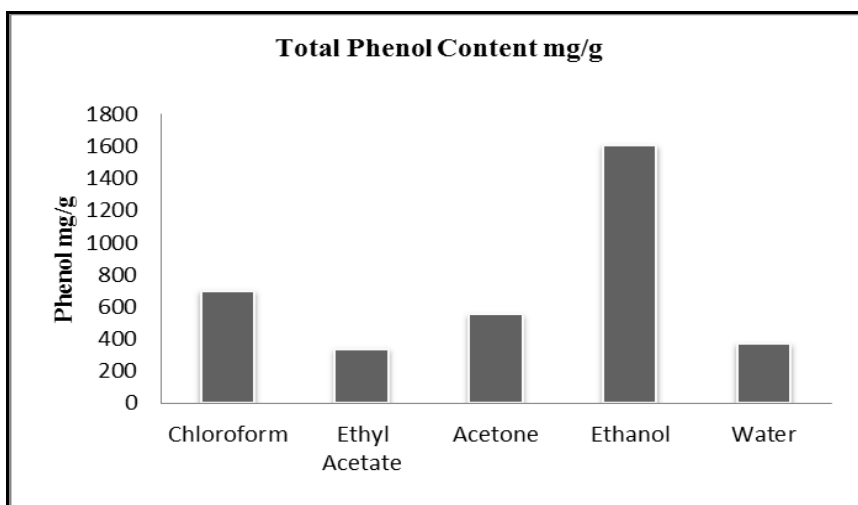
Quantitative determination of phenol and flavonoid of aerial extracts were performed using spectrophotometric method. The total phenol content was calculated as pyrocatechol equivalent and total flavonoid content was calculated as quercetin equivalent. Total phenol content of *A. cruentus* is obtained from the regression equation of calibration curve of pyrocatechol ( $r^2=0.934$ ) and expressed as pyrocatechol equivalent. Total flavonoid content is obtained from the regression equation of calibration curve of quercetin ( $r^2=0.993$ ) and expressed as quercetin equivalent. Plant extracts with a high phenol content also enclosed high flavonoid content. The amount of phenolic and flavonoid content of *A. cruentus* are recorded in (Table 1 and 2, Graph 1 and 2).

**Table 1: Total Phenol Content of Extracts**

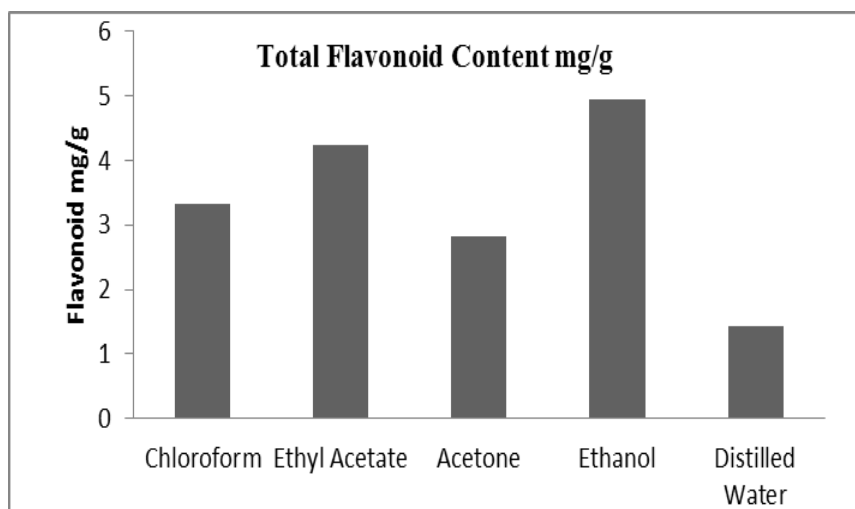
Total Phenol Contents mg/g $\pm$ SEM		
1	Chloroform	700.95 $\pm$ 0.04
2	Ethyl Acetate	341.47 $\pm$ 0.05
3	Acetone	500.95 $\pm$ 0.07
4	Ethanol	1611.66 $\pm$ 0.06
5	Water	380.64 $\pm$ 0.04

**Table 2: Total Flavonoid Content of Extracts**

Total Flavonoid Contents mg/g $\pm$ SEM		
1	Chloroform	3.40 $\pm$ 0.04
2	Ethyl Acetate	4.70 $\pm$ 0.05
3	Acetone	2.80 $\pm$ 0.07
4	Ethanol	4.95 $\pm$ 0.04
5	Water	1.44 $\pm$ 0.04



**Graph 1: Total Phenol Content of Extracts**



**Graph 2: Total Flavonoid Content of Extracts**

## Conclusion

This study indicates that the ethanol extract obtained from aerial part of medicinally important plant *Amaranthus cruentus* contain high amount of phenol and flavonoid compounds. This preliminary study is certainly useful to do further biological study.

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## References

1. Harborne J. B. Introduction to Ecological Biochemistry, 2<sup>nd</sup> Ed. Academic Press, New York, NY,1982
2. Harborne J. B., Turner B. L., Plant Chemosystematics, Academic Press, London, UK, 1984
3. Pridham J. B. Phenolics in Plants in Health and Disease, Pergamon Press, New York, NY,1960
4. Shahidi F., Naczki M., Phenolics in Food and Nutraceuticals Sources, Applications and Health Effects, CRC press,Boca Raton, FL,2004
5. Beckman C. H., Physiology, Molecular Plant Pathology,2000,57,101 -110
6. Wu H., Haig T., Prately J., Lemerie D., An M., Journal of Chromatography A 1999, 864,315-321
7. Wu H., Haig T., Prately J., Lemerie D., An M., Journal of Agricultural Food Chemistry, 2000,48,5321-5325
8. Einhellig F. A.,Putnam A. R.,Tang C. S., The Science of Allelopathy, John Wiley and Sons, New York, 1996, 171- 189
9. Brenda W S, Flavonoids in seeds and grains: Physiological function, agronomic importance and the genetics of biosynthesis, Seed Science Research, 1998, 8, 415-422.
10. Middleton E and Kandaswami C, The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer, in the flavonoids, Advances in Research Science (Ed.) Harborne, I R , Chapman and Hall, London, 1993, p; 619-645.
11. Winkel-Shirley, Biosynthesis of flavonoids and effects of stress, Current OpinionPlant Biology, 2002, 5, 218-223
12. Lamson D.W. and Brignale M S Antioxidants and cancer III: quercetin, Alternative Medicine Review, 2000, 5 (3), 196-208.
13. González R., Tosi E., Ré E., Añón M.C., Pilosof A.M.R., Martinez K. Amaranth starch-rich fraction properties modified by high-temperature heating. Food Chemistry, 2007, 103, 927–934.
14. Tosi E.A., Ré E., Lucero H., Masciarelli R., Dietary fiber obtained from amaranth (*Amaranthus cruentus*) grain by differential milling. Food Chemistry, 2001, 73, 441-443.

15. Fasuyi A.O. Bio-nutritional evaluations of three tropical leaf vegetables (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) as sole dietary protein sources in rat assay. *Food Chemistry*, 2007, 103, 757-765.
16. Fasuyi, A.O. Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. *African Journal of Biotechnology*, 2006; 5, 049-053.
17. Odhav B.; Beekrum S.; Akula U.; Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa *Journal of Food Composition Analysis*, 2007, 20,430-435.
18. Aletor V.A.; Adeogun O.A. Nutrient and anti-nutrient components of some tropical leafy Vegetables, *Food Chemistry*, 1995, 53, 375-379.
19. Guerra-Matias, A.C.; Arêas J.A.G., Glycemic and insulinemic responses in women consuming extruded amaranth (*Amaranthus cruentus* L.). *Nutritional Research*, 2005, 25, 815-822.
20. Cai Y.; Sun M.; Corke, H. Characterization and application of betalain pigments from plants of the *Amaranthaceae*. *Trends Food Technology*, 2005, 16, 370-376.
21. Cai, Y.; Corke, H. *Amaranthus* Betacyanin pigments applied in model food systems. *Journal of Food Science* 1999, 64, 869-873.
22. Nacoulma O.G. Millogo R. J., Vandamme P., *Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: casdu plateau central*; Thèse d'état, Université de Ouagadougou, 1996, p. 574.
23. Dhellot J.R.; Matouba E.; Maloumbi M.G.; Nzikou J.M.; Safou Ngoma D.G.; Linder M.; Desobry S.; Parmentier M., Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (var 1 and 2) of Congo Brazzaville. *African Journal of Biotechnology*, 2006, 5, 1095-1101.
24. Hilou, A. Etude phytochimique et activités biologiques d'extraits de deux Caryophyllales à bétalaïnes; *Amaranthus spinosus* L. (*Amaranthaceae*) et *Boerhaavia erecta* (*Nyctagynaceae*), plantes médicinales du Burkina Faso. Thèse unique de doctorat, Université de Ouagadougou, 2006, p.176
25. Malik E.P, Singh M. B., *Plant Enzymology and Hittoenzymology* 1<sup>st</sup> ED. New Delhi Kalyani Publishers, 1980.
26. ChangC., YangM., WenH., Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods , *Journal of Food and Drug Analysis*, 2002, 10, 178-182.

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