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Antioxidant Potential in Leaf and Stem Extracts of Different Amaranth Cultivars

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Abstract:- Amaranth phenolic and anthocyanins have a powerful biological activity, which attracts scientists for their investigation. In this present study, the total phenolic content in leaves and stems of different amaranth cultivars was determined by Folin- Ciocalteu method using Gallic acid as standard, And the quantification of the total anthocyanin was performed by microplate spectrophotometric method.

The results showed a higher quantity of antioxidant polyphenols in leaves than in stems. The total phenolic content ranged from 65.7677 ± 6.63to 542.323 ± 5.57 µg / ml of extract and total anthocyanin content from 0.054632 to 1.15832%. Among the total cultivars of amaranth studied, the highest concentration of anthocyanins was found in A. cru PT in both leaves and stems and the highest total phenolic content in leaves of A. hypo P and stems of A. hyb O; while the highest amino acid content observed in leaves of A. cru HRD and stems of A. hyb O. It was clearly observed that cultivars of *amaranthus caudatus* had shown lower antioxidant potential than others and the results revealed that all amaranth cultivars analyzed in this study were the source of phenols in different proportion within the cultivar.

***Keywords**: Amaranth Cultivars, Anthocyanin, Gallic Acid, Total Phenolic Content.*

I. INTRODUCTION

Antioxidants are substances that reduce the effect of free radicals and inhibit oxidation [1]. The antioxidant potential has been attributed to the presence of appreciable levels of phenolics and flavonoids [2]. Leaves, flowers, and other aerial parts (stalks) of Amaranthus as well as their extracts were shown to possess the highest antioxidant activities compared to other parts [3]. From a practical point of view, these antioxidants may also be used to counteract the deterioration of stored food products [4].

The recent findings of [3] reported that amaranth is a gluten-free pseudo cereal and a fast-growing plant available most of the year cultivating in all temperate-tropical areas of the world. Amaranth leaves and stems are inexpensive (cheap vegetables) and excellent sources as well as good economic sources of carotenoids, proteins, including the essential amino acids methionine and lysine, dietary fiber, and minerals, such as magnesium, calcium, potassium, copper, phosphorus, zinc, iron, and manganese [5,6,7&8]. They are also abundant in several pigments, such as carotenoids, chlorophylls, amaranthine, anthocyanins, betalains, betaxanthins, and betacyanins, and natural antioxidant phytochemicals, such as vitamin C, beta-carotene, flavonoids, and phenolic acids [5, 6, 7 & 8].

Anthocyanins are a group of water-soluble blue, purple, and red-colored pigments [9]. In the industry, there is a growing interest in these flavonoids due to their potential as a natural colorant and antioxidant capacity. They may be present in all tissues of the plant, including leaves, stems, roots, flowers, and fruits, and accumulate gradually during development. The anthocyanins content varies widely depending on the species, variety, environmental factors and growth, and storage conditions [9]. The maximum content is observed at harvest time and, during senescence, there is a reduction of this pigment [9].

Amaranth extracts act as traditional medicinal plants, especially as antiviral, antimalarial, anti-diabetic, antibacterial, anti-helminthic, and snake antidotes [5&6]. These compounds give protection against several diseases including neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, emphysema, retinopathy, atherosclerosis, and arthritis. These compounds also have a significant role in promoting the health-benefit and food colorants [7&8]. However, the literature has shown that the amaranth leaf had much higher nutrients, minerals, pigments, phytochemicals, and antioxidants in comparison to the stem of the plant [3&10]. For this reason, we evaluated the leaf and stem amaranth in terms of antioxidants such as phenols and anthocyanins. So the present study was conducted to quantify the total phenolic content and anthocyanin concentration in different amaranth cultivars.

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II. MATERIALS AND METHODS

➢ *Collecting of Amaranth Leaves and Stems*

After collecting the seeds from the Botanical garden of Ural Federal University, amaranth leaves and stems used were grown indoor controlled pots in the biological laboratory of the Institute of natural sciences and mathematics, Ural Federal University. After a month and a half, the healthy leaves and the stems of nine amaranth cultivars were harvested and dried in an oven at 80°c.

Table 1 Identification of Amaranth cultivars used

No	Species	Cultivar	Origin	Registration number	Abbreviation
1	*Amarantus caudatus L.*	cv. Edulis	Germany	49406-16	A.ca Ed
		f. Yellow brown	Germany	45378-16	A.ca Yb
		R-124	Austria	28893-95-05-16	A.ca R-124
2	*Amarantus cruentus L*	cv. Hopi Red Dye	France	29844-97-04	A.cru HRD
		cv. Nodoja	Romania	44628-09-10-16	A.cru N
		cv. Pygmy &Torch	Romania	49471-16	A.cru PT
3	*Amarantus hybridus L.*	cv. Oeschberg	Germany	41398-03-08-12-16	A.hyb O
4	*Amarantus hypochondriacus L.*	Unknown	Poland	49785-18	A.hypo P
		cv. Black leaved	Germany	47668-16	A.hypo Bl

➢ *Amaranth Leaves and Stems Extraction*

The dried leaves and stems were ground to a fine powder. 150 mg of dry leaves and stems material were accurately weighed out and extracted with 3 ml of 80% ethanol followed by shaking and incubating by ultrasonic water bath at 45°c temperature for 20 minutes. The amaranth extracts were then centrifuged at 10000 rpm for 10 minutes, and the supernatants were transferred to the new test tubes. The pellets were similarly extracted with 3 ml of 60% ethanol, and also the supernatants were kept in the same test tubes. At the third step, the pellets were similarly extracted with 3 ml of 40% ethanol and the supernatants were taken. Finally, the pellets were similarly extracted with 3ml of distilled water to keep the supernatants.

➢ *Determination of Total Phenolic Content*

Total phenolic content was determined with Folin-Ciocalteau assay [11], using Gallic acid as a standard phenolic compound. The standard stock solution was prepared by dissolving 100 mg of Gallic acid in 10 ml of 80% ethanol and then making the volume to 100 ml with distilled water in a 100 ml volumetric flask to get the concentration of 1 mg/ml (1000µg/ml). The calibration standard solutions were prepared by diluting the primary stock solution with distilled water to get calibration standard solutions of 100, 200, 400, 600, 800, and 1000µg/ml of Gallic acid solution, the absorbance was measured at λmax 760 nm, against reagent blank to construct standard plot curve (Fig1 &Table2). Briefly, 0.1ml of each plant extract (leaf and stem) solution was taken in Eppendorf tubes (three replicates), and 0.5 ml of Folin- Ciocalteau solution was added and mixed for 5 minutes .0.4 ml of 7.5% sodium carbonate solution was added to the mixture. The solution was mixed and then incubated for 1 hour in the dark. After that, the samples were centrifuged at 12000 rpm for 15 minutes. After centrifugation, the absorbance against the prepared reagent blank was determined at 760 nm. Test samples were analyzed in triplicate and the concentration of the sample was determined using the calibration curve. The mean value is reported in Gallic acid equivalents (GAE) using units of µg/mg of extract.

➢ *Determination of anthocyanin*

The anthocyanin concentration in eight amaranth cultivars was extracted using the technique of water-based extraction known as acidified water [12], 10 ml of hydrochloric acid (HCl 1%) was added to 400 mg of leaf/ stem dry biomass (two replicates for each sample) and incubated in dark place 24 hours. This solution was heated using an ultrasonic water bath at 45°c for about 25 minutes. We then centrifuged them at 10000 rpm for 15 minutes and filtered the supernatant. The absorbance was measured spectrophotometrically at 510 nm and 657 nm. Final the concentration of anthocyanin was calculated using the formula below:

$$\mathbf{CA=(Absorbance\ 510-Absorbance\ 657)Volume\ of\ HCL\ in\ liter\ (453*mass\ of\ sample\ in\ gram)}$$

III. RESULTS

➢ *Calibration curve construction and total phenolic content calculation in different amaranth cultivar extracts*

The method of Folin-Ciocalteu using Gallic acid as the standard was used to determine the total phenolic content in nine amaranth cultivar extracts. The absorbance values obtained at different concentrations of Gallic acid (Table 2) were used for the construction of the calibration curve (Fig 1). Total phenolic content of amaranth cultivar extracts was calculated from the regression equation of calibration curve, $Y = 0.0033x + 0.0416$, $R^2 = 0.9989$, and expressed as µg Gallic acid equivalents (GAE) per ml of sample in extract solution (µg/ml). The results are presented in Table 3.

Table 2 Absorbance of Gallic Acid for Calibrating both leaf and Stem Phenol Concentration

Concentration of Gallic acid [µg/ml]	Absorbance at 760 nm
1000	3.3106
800	2.7121
600	1.9701
400	1.3378
200	0.7028
100	0.4303

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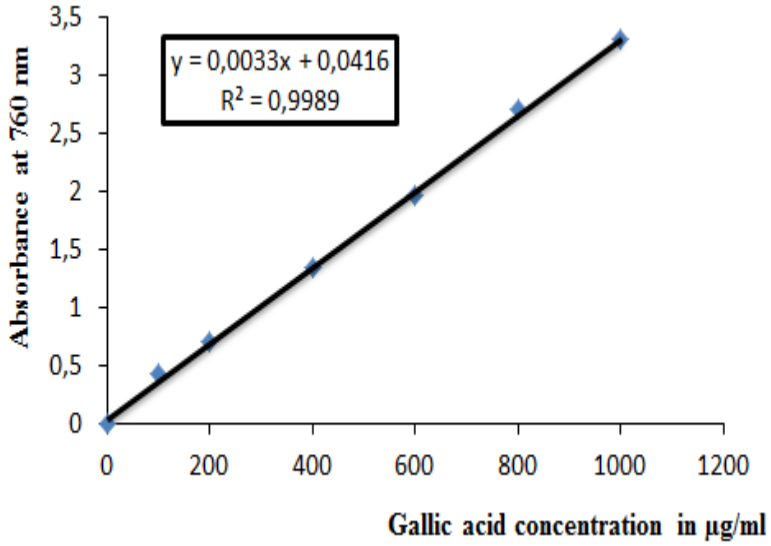

Fig 1 Calibration Curve of Gallic acid

Table 3 The means and standard errors of leaf and stem phenolic content calculated from their corresponding absorbance

No.	Plant	Leaf Abs (Mean ± SE)	Stem Abs (Mean ± SE)	Leaf Phenol conc. (Mean ± SE) in µg/ml	Stem Phenol conc. (Mean ± SE) in µg/ml
1	A.ca R-124	1.0249 ± 0.05	0.46243 ± 0.01	297.97 ± 1.21	127.525 ± 2.50
2	A.ca Ed	1.08583 ± 0.10	0.35413 ± 0.002	316.434 ± 3.01	94.7071 ± 0.66
3	A.ca Yb	1.26927 ± 0.06	0.38547 ± 0.011	372.02 ± 1.65	104.202 ± 3.23
4	A.cru HRD	1.52167 ± 0.05	0.25863 ± 0. 022	448.505 ± 1.26	65.7677 ± 0.63
5	A.cru N	1.66323 ± 0.15	0.45533 ± 0.025	491.404 ± 4.08	125.374 ± 0.42
6	A.cru PT	1.61997 ± 0.11	0.57957 ± 0.01	478.293 ± 3.58	163.02 ± 2.43
7	A.hyb O	1.68107 ± 0.05	0.6684 ± 0.029	496.808 ± 1.74	189.939 ± 0.87
8	A.hypo P	1.83127 ± 0.02	0.43127 ± 0.021	542.323 ± 5.57	118.081 ± 0.49
9	A.hypo Bl	1.4453 ± 0.03	0.52417 ± 0.01	425.364 ± 1.18	146.232 ± 1.98

P value and regression statistical between leaf phenolic content and stem phenolic content, P = 0.371, R = 0.34, and R^2= 0.12

The total phenolic content of leaf extract ranged from 297.97 ± 1.21 to 542.323 ± 5.57 µg GAE/ ml (g) extract (Table 3). The ascending order of the total phenolic content values are as 297.97 ± 1.21 ; 316.434 ± 3.01 ; 372.02 ± 1.65 ; 425.364 ± 1.18 ; 448.505 ± 1.26 ; 478.293 ± 3.58 ; 491.404 ± 4.08 ; 496.808 ± 1.74 and 542.323 ± 5.57 µg GAE/ ml (g) extract for A. ca R-124 ; A. ca Ed ; A.ca Yb ; A.hypo Bl ; A.cru HRD ; A.cru PT ; A.cru N ; A. hyb O and A.hypo P respectively (Table 3). The cultivars of the same species have represented the neighboring values of total phenolic content with an exception for A.hypo Bl . The lowest phenolic content was noted for the cultivars of A. *caudatus* , followed by one cultivar as an exception in our assumptions, A.hypo Bl, then the middle phenolic content for the cultivars of A.*cruentus* and the highest for the cultivars of A.*hybridus* and *hypochondriacus L.*

The amaranth cultivar stem extracts have shown the lowest phenolic content compared to the amaranth cultivar leaf extracts. The values of total phenolic content in stem extracts ranged from 65.7677 ± 0.63 to 189.939 ± 0.87 µg GAE/ ml (g) extract (Table 3). However the cultivars are ordered as follow from the lowest to highest total phenolic content, 65.7677 ± 0.63 ; 94.7071 ± 0.66 ; 104.202 ± 3.23 ; 118.081 ± 0.49 ; 125.374 ± 0.42 ; 127.525 ± 2.50 ; 146.232 ± 1.98; 163.02 ± 2.43 and 189.939 ± 0.87 µg GAE/ ml (g) extract for A.cru HRD ; A.ca Ed; A.ca Yb; A.hypo P ; A.cru N ; A.ca R-124 ; A.hypo Bl ; A.cru PT and A.hyb O respectively (Table 3). This proper order of stem phenolic content showed clearly that there is no connection between the cultivars of the same species which is a dissimilar observation with leaf phenolic content. Thus the results revealed that there is no correlation between the stem phenolic content and leaf phenolic content of the same species as well as the same cultivar (P = 0.371, R = 0.34, and R^2= 0.12).

➢ *Anthocyanin concentration*

The results showed that the anthocyanin concentrations between amaranth cultivars were different and they were high in leaves than in stems within the same cultivar. They ranged from 0.26872 to 1.15832% in leaves and from 0.054632 to 0.266817% in stems. Moreover these values in both leaves and stems corresponded from A. hyb O to A. cru PT. In turn, for both leaves and stems, only the cultivars of *amaranthus caudatus* had the closer lowest concentrations and the proper classification is from 0.26872; 0.32924; 0.35995; 0.39982; 0.42211; 0.55936; 0.83107 and 1.15832% for A. hyb O ; A. ca Ed ; A. ca R-124 ; A. ca Yb ; A. hypo Bl ; A. cru N ; A. hypo P and A. cru PT respectively (in leaves).

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For stems, the concentrations ordered from 0.054632; 0.0559; 0.069581; 0.071846; 0.072571; 0.120136; 0.145594 and 0.266817% for A. hyb O ; A. ca Yb ; A. ca Ed ; A. ca R-124; A. hypo Bl ; A. hypo P ; A. cru N and A. cru PT respectively (Table 4).

Table 4 Anthocyanin concentrations in percentage in both leaves and stems of eight cultivars

No.	Plant	Leaf (A510–A657) Mean	Stem (A510–A657) Mean	Leaf anthocyanin conc. in %	Stem anthocyanin conc. in %
1	A.ca R-124	0.19865	0.03965	0.35995	0.071846
2	A.ca Ed	0.1817	0.0384	0.32924	0.069581
3	A.ca Yb	0.22065	0.03085	0.39982	0.0559
4	A.cru N	0.3087	0.08035	0.55936	0.145594
5	A.cru PT	0.63925	0.14725	1.15832	0.266817
6	A.hyb O	0.1483	0.03015	0.26872	0.054632
7	A.hypo P	0.45865	0.0663	0.83107	0.120136
8	A.hypo Bl	0.23295	0.04005	0.42211	0.072571

IV. DISCUSSION

➢ *Total phenolic content*

The total phenolic content in different amaranth cultivar extracts using ethanol and water solvent for both leaves and stems was determined. The lowest total phenolic content was noted from stem extracts than leaf extracts. A similar trend was reported by several studies, especially in three species A. *hypochondriacus* , *caudatus* and *cruentus* [3& 13]. Polyphenols are secondary metabolites such as phenolic acids, flavonoids, tannins, and anthocyanins that occur naturally in cereals, fruits, pseudo cereals, and vegetables [14]. [13] reported that these compounds vary among *Amaranthus species*, genotypes, and functional parts (stems, leaves, stalks, seeds..,). It is in this regard our present study matched. Previous several studies reported similar results to us, For example [2] found the values of total phenol content from 0.15 to 5.24 mg GAE /g sample, and [15] found the total phenol content of *amaranthus hybridus* leaves ranged from 145 to 583 mg GAE/ kg in spring and fall respectively. The low values of total phenol content than our values were ranged from 1.73 mg /100g GAE (0.0173 mg/g) to 2.29 mg / 100g GAE (0.0229mg/g) in the leaves of *amaranthus tricolor* L [16].

Our study reported the total phenol content in leaves from 297.97 to 542.323 µg GAE/ ml (g) extract (0.29797 to 0.542323 mg/g), this range is low to the total phenol content range in the study of [3], It ranged from 18.3 to 33.7 mg GAE /g extract for aerial parts of *amaranthus caudatus* in the flowering stage and the TPC on the basis of plant fresh matter, ranged from 0.68 to 1.11 mg/g and lastly in the early flowering stage, TPC of *amaranthus caudatus* extract from separate leaves ranged from 24.8 to 32.3 mg GAE /g extract. The total phenol content in stems in this present study ranged from 65.7677 to 189.939 µg GAE/ ml (g) extract (0.0657677 to 0.189939 mg/g). This range is low according to [13] found the TPC ranged from 1.04 to 14.94 mg GAE / g DW where 1.04 mg GAE/ g DW is the value of *amaranthus* stalks and its phenolic compounds of 30 to 51 mg/ 100g DW. The phenolic composition in amaranth leaves; stalks and flowers has not been well collectively documented.

Several studies have reported that amaranth contains phenolic compounds such as rutin and quercetin [3]. This explains that high phenolic acids in leaves, flowers, stalks, and seeds of amaranth contain high phenolic compounds [13]. The phenols enhance antioxidant activity due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [17]. So the total phenolic content was significantly higher in leaves because they were exposed to the sunlight than stems and consequently they provide more antioxidant activity than stems. This argument came from the hypothesis said that the total phenolic content, as well as antioxidant activity, increased proportionally to the light intensity, which plays a strong role in the accumulation of phenols and antioxidants [17]. The light intensity affects not only the phytochemicals but also the plant morphological traits. Therefore total phenolic content is a good indicator for assessing antioxidant activity [17].

➢ *Total concentration of anthocyanin*

The fruits and vegetables are considered as good sources of this pigment. The extraction of anthocyanins with the use of acids reduces the risk to the manipulator and the contamination of the environment [9]. We extracted anthocyanin in leaves and stems using only hydrochloric acid while many authors demonstrated the anthocyanins extraction techniques using 70% ethanol with hydrochloric acid at pH 2.0, all anthocyanins are soluble in polar solvents, and the use of water added to the solvent facilitates the extraction of more hydrophilic anthocyanins [9]. Our results as expressed in percentage, the leaves extract represented a high anthocyanin content than the stems extract and they ranged from 0.26872 to 1.15832% and 0.054632 to 0.266817% respectively. These results are very high compared to [18] with the total anthocyanin content of amaranth leaves ranging from 0.080 – 8.727 mg/g and in stem-peel TAC was in the range of 0.84 -2.5067 mg/g. Similar to our findings, in their study, the mean TAC value in inflorescence was greater followed by leaves and then stem-peel.

Solvent acidification increased anthocyanins extraction efficiency for some fruits and vegetables. The increase in anthocyanin content, suggests that denaturation of cell membranes, either by the physical or chemical constitution, is difficult in these species, or that the anthocyanins have more

strongly adhered to the vacuoles of the cells [9]. The large increase in the extraction with the acidification of the extraction solvent has already been demonstrated by [20]. Moreover, the stability of the anthocyanins increases with the decrease of the pH of the medium.

Acidification may be necessary to promote denaturation of cell membranes with subsequent release of anthocyanins present in the vacuole of some plant species [21] however, it is necessary to know the behavior of different species for efficient extraction. It has been reported that the extraction of acylated anthocyanins under acidic conditions may cause their total or partial hydrolysis [22] and the extraction efficiency may be related to the type of acid used [23]. Therefore, the use of acidified solvents to extract anthocyanins can produce proanthocyanidins and flavonols, which may result in an overestimation of the total anthocyanins. [9] reported total anthocyanin content in the phenolic extracts varied from 0.22 to 365.86 mg·100 mL-1 of extract in the 13 studied fruits and vegetables. The same [19] reported the anthocyanins concentration in plum peels (60.45 mg/100g), blueberry (22.38 mg/100g), and apple peels (16.39 mg/100g). All of these findings in fruits are low than those obtained with us. Anthocyanin pigments play an important function in plant physiology as they play a major role in pollination, and seed dispersal and also protect the leaf from UV light [24]. The role of anthocyanin pigments as medicinal agents has been well-accepted dogma in folk medicine throughout the world and has multifaceted roles in human health maintenance [18]. In comparison with the total anthocyanin content of fruits, vegetables, and juices; leaves and stems of different amaranths in the present study recorded a higher anthocyanin pigment and thus can also be utilized as a potential source of this natural pigment.

V. CONCLUSION

It can be concluded that the quantity of the phenolic compounds namely total phenol content and total anthocyanin content in amaranth varies across the plant parts (the leaves showed higher content than stems) and cultivar. In general, the values of total phenolic content were normal with other findings whereas the anthocyanin concentration values were higher. The amaranth cultivars investigated in this study showed the presence of diverse phenolic compounds. These phenolic compounds have health benefits for human beings which may aid in fighting against different diseases. Anthocyanins are value-added colorants that can be used for preventing several diseases, including CVDs, cancers, diabetes, some metabolic diseases, and microbial infection. These compounds also improve visual ability and have a neuroprotective effect. Therefore the results obtained from this study indicate the importance of amaranth as a promising source of phenolic compounds that will help in developing new opportunities for their use in the food and pharmaceutical industries and can be utilized as a natural source of anthocyanin pigment and can be proposed to be part of the production of eco-friendly products. However, future studies are recommended for individual compound purification and their mechanisms of action which will provide a better understanding of antioxidant activity nature before they can be included in human diets and used in the industrial manufacture of drugs.

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