

ANTI-NUTRIENT AND PHYTOCHEMICAL EVALUATION OF 10 AMARANTH VARIETIES BEFORE AND AFTER FLOWERING

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Abstract

Amaranth vegetable is widely consumed in Kenya and contributes to the alleviation of food insecurity. It is reported to have bioactive components such as antioxidants that help protect the body from long-term degenerative diseases. However, amaranth vegetable has also been shown to contain some anti-nutrients such as tannins, phytic acid, oxalates and nitrates which may bind nutrients and reduce their bioavailability in the body. There are many amaranth varieties and there is very little information about differences in the anti-nutrient and phytochemical levels among the varieties. The objective of this study was to determine the phytochemical and anti-nutrient content of ten amaranth varieties at two growth stages, before and after flowering. The study was in JKUAT. Ten amaranth varieties, eight of which are new varieties, were planted. Harvesting was done in two stages, at vegetative stage and post flowering stage. The leaves were analyzed for nitrates, tannins, vitamin C, total carotenoids, flavonoids and total antioxidant activity. Results showed increase in most anti-nutrients, except nitrates which reduced by about 40% as the plants matured. The leaves also increased in the total antioxidative activity as the plants matured with the concentration equivalent (IC₅₀) with DPPH of 2mg/ml at vegetative stage and about 1mg/ml after flowering. In conclusion, as the plants grow older, there was increased accumulation of antinutrient and other plant chemicals. Antioxidative activity is however increased.

Key words: Amaranth, malnutrition, anti-nutrients, phytochemicals, antioxidant activity.

1.0 Introduction

Amaranth (*Amaranthus spp.*) is one of the Africa leafy vegetables from the family *Amaranthaceae*, and is believed to have originated from Africa, the Americas and Europe. It is a herbaceous annual crop cultivated for both its leaves which are used as a vegetable and its seeds which are used as a grain. It is also often found growing wildly. Vegetables including amaranth are an important component of the diet as they provide important nutrients and also have bioactive components such as antioxidants that help protect the body from long-term degenerated diseases (Raheena, 2007). However, the vegetable amaranth has also been shown to contain some anti-nutrients such as tannins, phytic acid, oxalates and nitrates which bind nutrients and may reduce bioavailability of the nutrients in the body.

Amaranth, just as other African Leafy Vegetables, has been a staple food to many African and Kenyan households for a long time. Some of its vernacular names in Kenya include *Ododo* (Luo), *Mchicha* (Swahili) *Terere* (kikuyu) *Chimboka* (luhya), *Ekwala* (teso), *Lookwa* or *Epapes* (turkana); (Jacob 2005).

Approximately 60 species are recognized, with inflorescences and foliage ranging from purple and red to green or gold. They produce edible leaves while others produce grains as well (Grubben 2004). They are also used as ornamentals while others are just weeds (He, 2002; Kauffman and Weber, 1990). Many varieties and cultivars of amaranth, both vegetable and grain are in existence. Further, new varieties, with unknown phytochemical and anti-nutritional characteristics are still being produced. The objective of this study was to determine the phytochemical and anti-nutrient content of ten amaranth varieties at two growth stages, before and after flowering.

2.0 Materials and Methods

2.1 Research Site

The study was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) in the year 2013.

2.2 Experimental Design and Treatments

The experiment was laid in randomized complete block design (RCBD) and was replicated three times. The treatments comprised ten different amaranth varieties, which were harvested at two growth stages, the vegetative stage and post flowering stage.

2.3 Crop Establishment

The ten varieties were planted on a 10 m by 5 m plot in JKUAT farm, in rows of 50 cm apart. Blocks were 2 m long and the distance between blocks was 1 m. Rainfall and irrigation were the sources of water for the crops. In the weeks where there was no rain, the plants were irrigated twice a week. Shallow cultivation using Jembes and hand pulling of weeds was carried out to keep the plot weed free.

2.4 Harvesting/ Sample Collection

Leaves were harvested at the vegetative stage, four weeks after planting and after flowering at about seven weeks after planting. The leaves were harvested by hand plucking and were then put in polythene bags and stored in a cold room awaiting laboratory analysis.

2.5 Phytochemical and Anti-Nutrient Analysis

Analysis was done on the anti-nutrient content (nitrates, tannins), vitamin C, carotenoids, flavonoids and total anti-oxidative activity.

2.6 Determination of Nitrates

The nitrate content in the test samples was determined by the calorimetric method using salicylic acid according to Cataldo *et al.*, (1975). A 500 mg of fresh leaves was weighed and put in a tube. Ten ml of hot (90-95°C) distilled water was added. The closed tubes were placed in a water bath at 80°C for 30 minutes and shaken. The samples were cooled and centrifuged at 4500 rpm. Supernatant was decanted and weighed to determine the exact volume of extract. Chlorophyll in leaves was removed by adding 0.5 g MgCO₃ to the supernatant, and centrifuged again. The supernatant containing the nitrate extract was treated with 2 N NaOH and a combination of Salicylic acid and H₂SO₄. Nitrate standards were also prepared using sodium nitrate calibration curve. Absorption was measured at 410 nm with UV spectrophotometer.

2.7 Determination of Tannins

Tannin content was determined by the Folis-Denis calorimetric method described by Kirk and Sawyer (1998).

2.7.1 Qualitative Analysis

About 0.5 g of dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 1.0% ferric chloride were then added. A brownish green or blue-black coloration indicated presence of tannins.

2.7.2 Quantitative Analysis

A 5 g of sample was ground in a mortar, dispersed in 50 ml distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28° C before it was filtered through Whatman NO. 42 filter paper. Various concentrations of standard tannic acid were made. Four ml of distilled water were placed in different test tube for samples and standards. To this, 1 ml of sample extract/standard tannic acid was added. To each, 0.5 ml of Folin Ciocateau solution (10 ml of 2 N Folin Ciocateau solution in 100 ml distilled water) was added. Then 1.5 ml of 7.5% Na₂CO₃ solution was added to each. This was then topped up with distilled water. Their respective absorbance was measured in a UV spectrophotometer at 720 nm using the reagent blank to calibrate the instrument at zero.

2.8 Determination of Vitamin C

The ascorbic acid content in the samples was determined by HPLC method according to Vikram *et al.*, (2005).

2.9 Determination of Total Carotenoids

Total carotenoids were extracted using acetone and analyzed using column chromatography and UV Spectrophotometer, (Rodriguez-Amaya and Kimura, 2004; AOAC, 1998).

2.10 Determination of Phytochemicals

The phytochemicals to be determined were flavonoids, total carotenoids and vitamin C. Additionally, the free radical scavenging activity was also determined. This activity results from the activity of the various phytochemicals.

2.11 Sample Extraction

About 5 g of dried and crushed samples were weighed into a 250 ml conical flask and about 100 ml methanol added. The flask was closed securely using parafilm and covered with aluminum foil. The samples were put in a shaker and shaken for about 3 hours. They were then kept in the dark and left to extract for 72 hours.

After 72 hours, the samples were filtered through Whatman No. 4 filter paper, and then the filtrate concentrated in a vacuum evaporator to a volume of 20 ml. The extract was transferred into vial bottles and securely stoppered. This extract was used for analysis of flavonoids and anti-oxidative activity.

2.12 Determination of Flavonoids

2.12.1 Qualitative Analysis

This was done according to the method of Harborne (1973); 5 ml of dilute ammonia solution was added to a portion of aqueous filtrate of extract followed by addition of concentrated H₂SO₄. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing.

2.12.2 Quantitative Analysis

Aluminum chloride colorimetric method was used for determination of flavonoids (Jagadish *et al.*, 2009). To 10 ml volumetric flask 4 ml of distilled water and 1 ml of plant extract (10 mg/ml) were added. After 3 minutes, 0.3 ml of 5 % sodium nitrite solution was added. After 3 minutes, 0.3 ml of 10 % aluminum chloride was added. After 5 minutes, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 ml with water. Absorbance was measured at 415 nm. The percentage of total flavonoids was calculated from Calibration curve of standard prepared from quercetin.

2.13 Determination of the Free Radical Scavenging Activity

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm (Molyneux, 2003). The following concentrations of the extracts were prepared, 0.01, 0.1, 1.0, 2.0 and 5 mg/ml in methanol (Analar grade). Vitamins C were used as the antioxidant standard at concentrations of same as the extract concentrations. 1 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbances were read at 517 nm after 5 minutes. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \{(AB - AA)/AB\} \times 100$$

Where AB is the absorption of blank sample and AA is the absorption of tested extract solution.

The results were expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC₅₀) determined from a plot of % inhibition of DPPH versus concentration of extract.

2.14 Data Management and Analysis

The data was analyzed statistically using analysis of variance at 5% level of significance using the statistical software Genstat Release 14.1, and the means were separated using Duncan's Multiple Range Test (DMRT) to determine whether there was significant difference in anti-nutrients and phytochemicals among the varieties at the two harvest stages.

3.0 Results and Discussion

3.1 Qualitative Results of Tannins and Flavonoids

The preliminary phytochemical screening of the methanolic extract showed the presence of flavonoids. Qualitative test also showed the presence of tannins.

3.2 Tannin content in the Amaranth Samples

The tannin content of the amaranth varieties varied significantly among the varieties ($P \leq 0.05$). The tannin values ranged between 16.0-18.8 mg/100 g at vegetative stage and 20.4-24.9 mg/100 g at post flowering stage (Table 1). There was significant increase ($p \leq 0.05$) in mean tannin content from 17.2 mg/100g before flowering to a mean of 22.1 mg/100g after flowering. Tannins are polyphenols with molecular weight greater than 500 and they have the ability to precipitate proteins from aqueous solutions. Tannins bind proteins, hence interfering with their bioavailability. Other studies have also shown tannins as a phytochemical and having some antioxidant activity in the body.

Table 1: Anti-nutrients in 100 g of the Fresh Amaranth Leaves

Variety	Nitrates (mg)		Tannins (mg TAE)	
	H1	H2	H1	H2
1	175.1± 0.5 ^{cd}	101.3 ± 1.3 ^a	16.3± 0.1 ^{ab}	21.3±0.2 ^b
2	203.9± 1.2 ^f	110.6 ± 5.1 ^b	18.5± 0.7 ^c	23.4±0.1 ^d
3	193.7± 2.9 ^e	124.9 ± 0.2 ^d	16.1± 0.4 ^a	20.4±0.03 ^a
4	196.2± 0.3 ^e	121.6 ± 2.5 ^{cd}	18.1± 0.1 ^c	22.8±0.05 ^c
5	183.7± 2.9 ^d	133.0 ± 0.6 ^e	16.7± 0.7 ^{ab}	20.7±0.2 ^a
6	205.2± 0.7 ^f	139.1 ± 1.3 ^e	17.2± 0.1 ^b	21.3±0.1 ^b
7	170.5± 1.0 ^{bc}	120.9 ± 0.7 ^c	16.0± 0.3 ^a	21.6±0.5 ^b
8	166.4± 3.7 ^{ab}	110.1 ± 1.7 ^b	16.4± 0.4 ^{ab}	21.4±0.2 ^b
9	164.4± 2.6 ^a	120.4±1.3 ^c	18.8± 0.7 ^c	23.2±0.2 ^{cd}
10	170± 0.5 ^{abc}	137.7±1.1 ^e	18.4± 0.6 ^c	24.9±0.6 ^e
Mean	182.1	121.9	17.2	22.1

H1- vegetative stage, H2-post flowering stage, TAE- Tannic acid equivalent. Means within the same column with same superscripts were not significantly ($p \leq 0.05$) different. Values are presented as Mean± SD, n=3

3.3 Nitrate Content in the Amaranth Samples

Nitrate contents varied significantly among the varieties at both the vegetative and post flowering stages. There was significant decrease in the mean nitrate content from 182 mg/100 g at vegetative stage to 121 mg/ml at post flowering stage (Table 1). High level of nitrates when ingested can be converted to nitrite which can lead to cancer and metheamoglobinemia or blue-baby disease (Gupta *et al.*, 2000; Macrae, *et al.*, 1997; Oguchi *et al.*, 1996; Takebe and Yoneyame, 1997). Nitrates can, however, be removed from amaranth leaves by boiling them in water for five minutes and discarding the water (Ogbadoyi *et al.*, 2006).

3.4 Vitamin C content in the Amaranth Samples

Amounts of ascorbic acid increased greatly as the plants grew older, (Table 2) with variety 7 increasing by up to 163%; from 66.4 mg/100 g at vegetative stage to 175.9 mg/100 g at post flowering stage. Variety 1, which had the highest amount at vegetative stage, had the least increase of 30% up from 117.1 mg/100 g at vegetative stage. The overall amounts are similar to reported values of 135 mg/100 g (Abukutsa-Onyango, 2003). Consumption of 100 g raw amaranth leaves can provide up to 270% of vitamin C daily requirement. Vitamin C (ascorbic acid) is however highly sensitive to air, light, temperatures and is also soluble in water. Hence most of it, 57%- 78% has been reported to be lost during cooking of vegetables. Shorter cooking time is therefore recommended to avoid greater losses of this vitamin. Human beings cannot synthesize vitamin C, which is a very important antioxidant. Vitamin C

is an important co-factor in protein chemistry and has many other uses. It strengthens immune system, fights infections, makes collagen, keeping bones, skin and joints firm and strong, antioxidant, protecting against cancer and heart disease.

3.5 Total carotenoid content in the amaranth samples

The total carotenoids content in the samples ranged from 6.7-8.2 mg/100 g at vegetative stage and 3.3-4.8 mg/100 g after flowering, with significant differences ($p \leq 0.05$) among the varieties. There was a significant decrease in the total carotenoid content from a mean of 7.4 mg/ml at vegetative stage to a mean of 4.1 mg/ml at post flowering stage (Table 2). Carotenoids are yellow, red and orange pigments present in many fruits and vegetables. In the diet they act as powerful antioxidants and are believed to protect the body against free radical attack and hence reduce the incidence of cataracts, heart disease and certain cancers (Kumar and Rao, 2012).

Table 2: Vitamin C and carotenoid composition in 100 g fresh weight of the amaranth varieties at vegetative and post flowering stage

Variety	Vitamin C (mg)		Total carotenoids(mg)	
	H1	H2	H1	H2
1	117.1± 3.9 ^e	152.7± 4.1 ^a	7.7± 0.21 ^d	3.5± 0.11 ^b
2	74.8± 4.9 ^b	155.0± 3.6 ^{ab}	8.2± 0.37 ^f	3.5± 0.04 ^b
3	68.6± 0.7 ^a	151.6± 3.4 ^a	6.8± 0.13 ^a	3.3± 0.08 ^a
4	76.6± 0.6 ^b	150.1± 1.7 ^a	6.7± 0.19 ^a	4.6± 0.14 ^e
5	85.1± 3.5 ^{cd}	149.8± 4.8 ^a	7.8± 0.07 ^e	4.8± 0.01 ^e
6	82.1± 3.4 ^c	176.2± 0.4 ^c	7.6± 0.29 ^c	4.6± 0.02 ^e
7	66.4± 2.3 ^a	175.9± 1.7 ^c	7.1± 0.12 ^b	4.2± 0.24 ^d
8	88.8± 3.7 ^d	159.6± 1.1 ^b	7.8± 0.05 ^d	3.6± 0.07 ^b
9	85.5± 0.5 ^{cd}	150.9± 0.6 ^b	7.1± 0.21 ^b	3.9± 0.04 ^c
10	84.9± 1.1 ^{cd}	158.1± 0.5 ^b	6.9± 0.08 ^{ab}	4.1± 0.04 ^c
Mean	83.0	158.0	7.4	4.1

H1- vegetative stage, H2-post flowering stage; Means within the same column with same superscripts were not significantly ($p \leq 0.05$) different. Values are presented as Mean± SD, n=3.

3.6 Flavonoid Content in the Amaranth Samples

At vegetative stage, flavonoid content ranged between 2819.9-4284.0 mg/100 g quercetine equivalent (QE), and 1446.5-2330.1 mg/100 g QE at post flowering stage. There was significant decrease in the flavonoid content, from 3254 mg/100 g at vegetative stage to 2033 mg/100 g at post flowering stage (Table 3). These values are relatively lower compared to those reported by Olajire and Azeez (2011) which were 69.67mg/ g (6967 mg/100 g) in *Amaranthus caudatus* and much higher than those reported by Akubugwo *et al.* (2008). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-neoplastic, antiviral, antithrombotic and vasodillatory activities (Miller, 1996). The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the above actions, as oxidative damage is implicated in most disease processes.

3.7 Antioxidant activity/ Free Radical Scavenging Activity

The lower the IC₅₀ value the higher the FRSA/antioxidant power. At vegetative stage, there was no significant difference ($p \leq 0.05$) in the antioxidant activity of seven varieties. Variety 8 had significantly higher antioxidant activity (lowest IC₅₀ value, 1.9 mg/ml) than other varieties but was not significantly different ($p \leq 0.05$) with variety 4 and 9 at 2.2 mg/ml. At post flowering stage, there was a significant rise in the antioxidant activity of all the vegetables to a mean IC₅₀ of 1.2 mg/ml. There was no significant difference ($p \leq 0.05$) among nine varieties. However, variety 2 had significantly lower antioxidant activity than variety 3 and 10. The leaf extract of amaranth

exhibited a good potential to act as a free radical scavenger. However the IC₅₀ for DPPH inhibition for amaranths (1.0-2.5 mg/ml) was comparably lower to that of Vitamin C (0.004 mg/ml) which is a known free radical scavenger.

Table 3: Flavonoid content as quercetin and total antioxidant capacity of the dried amaranth leaves

Variety	Flavonoid (mg/100g QE)		Antiox activity: IC ₅₀ (mg/ml)	
	H1	H2	H1	H2
1	4284.0± 19.2 ^g	2330.1± 2.8 ^f	2.3± 0.17 ^b	1.2± 0.1 ^{ab}
2	3368.1± 4.9 ^e	1929.5± 12.5 ^c	2.3± 0.29 ^b	1.5± 0.4 ^b
3	3629.9± 15.3 ^f	1697.5± 13.2 ^b	2.5± 0.36 ^b	1.0± 0.05 ^a
4	3026.5± 5.6 ^b	1975.9± 16.1 ^c	2.2± 0.17 ^{ab}	1.3± 0.2 ^{ab}
5	3170.1± 7.0 ^c	2279.5± 20.7 ^e	2.4± 0.11 ^b	1.2± 0.0 ^{ab}
6	3002.9± 2.7 ^b	2065.7± 10.4 ^d	2.3± 0.15 ^b	1.3± 0.05 ^{ab}
7	2819.9± 12.5 ^a	2198.7± 17.3 ^d	2.3± 0.2 ^b	1.2± 0.0 ^{ab}
8	3201.0± 8.4 ^d	2298.7± 10.4 ^e	1.9± 0.2 ^a	1.4± 0.2 ^{ab}
9	3048.9± 4.9 ^b	1446.5± 10.3 ^a	2.2± 0.15 ^{ab}	1.1± 0.3 ^{ab}
10	2994.8± 7.6 ^a	2117.5± 10.3 ^d	2.5± 0.2 ^b	1.0± 0.1 ^a
Mean	3254.6	2033.9	2.3	1.2

H1- vegetative stage, **H2**- post flowering stage, **IC₅₀**- concentration at 50% DPPH inhibition, **QE**- quercetin equivalent. Means within the same column with same superscripts were not significantly ($p \leq 0.05$) different. Values are presented as Mean± SD, n=3.

High % inhibition of DPPH is an indication of high free radical scavenging activity (FRSA) of the extract. The methanol extracts of the amaranth varieties showed potential of free radical scavenging activity against DPPH, which was, however, considerably lower compared to vitamin C as a free radical scavenger.

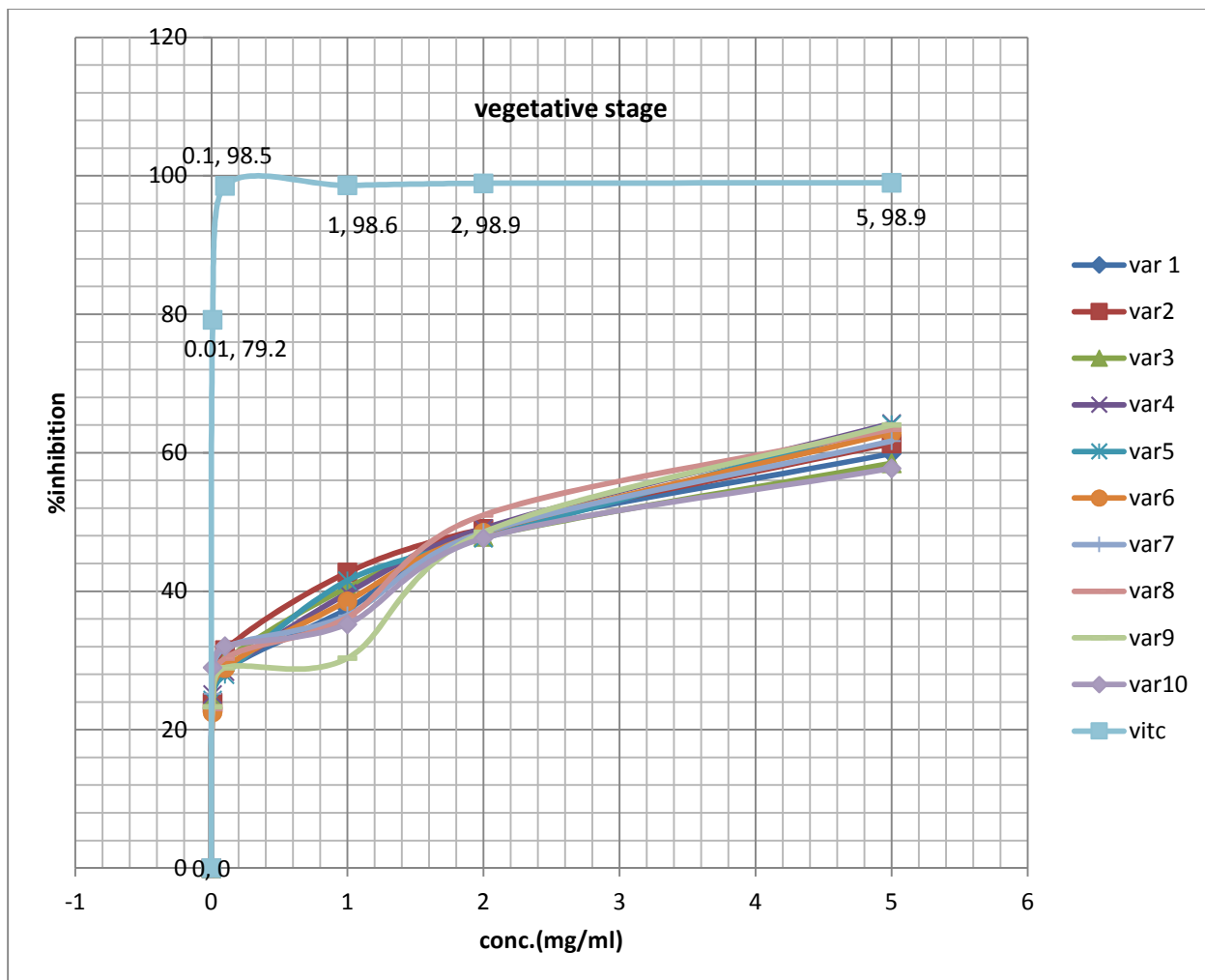


Figure 1: Inhibition (%) of DPPH against concentration of extracts of amaranth leaves harvested at vegetative stage and Vitamin C

At vegetative stage, maximum inhibition was achieved at a high concentration of 5 mg/ml (57.7-64.2%) for the amaranth leaves compared to 0.1 mg/ml (98%) for Vitamin C (Figure 1). At post flowering stage, maximum inhibition was achieved at extract concentration of 5 mg/ml (63.1-71.8%) in the different varieties, and at 0.1 mg/ml (98%) for vitamin C (Figure 2). Hence a higher concentration of amaranth leaves will be required to achieve maximal inhibition of DPPH compared to Vitamin C.

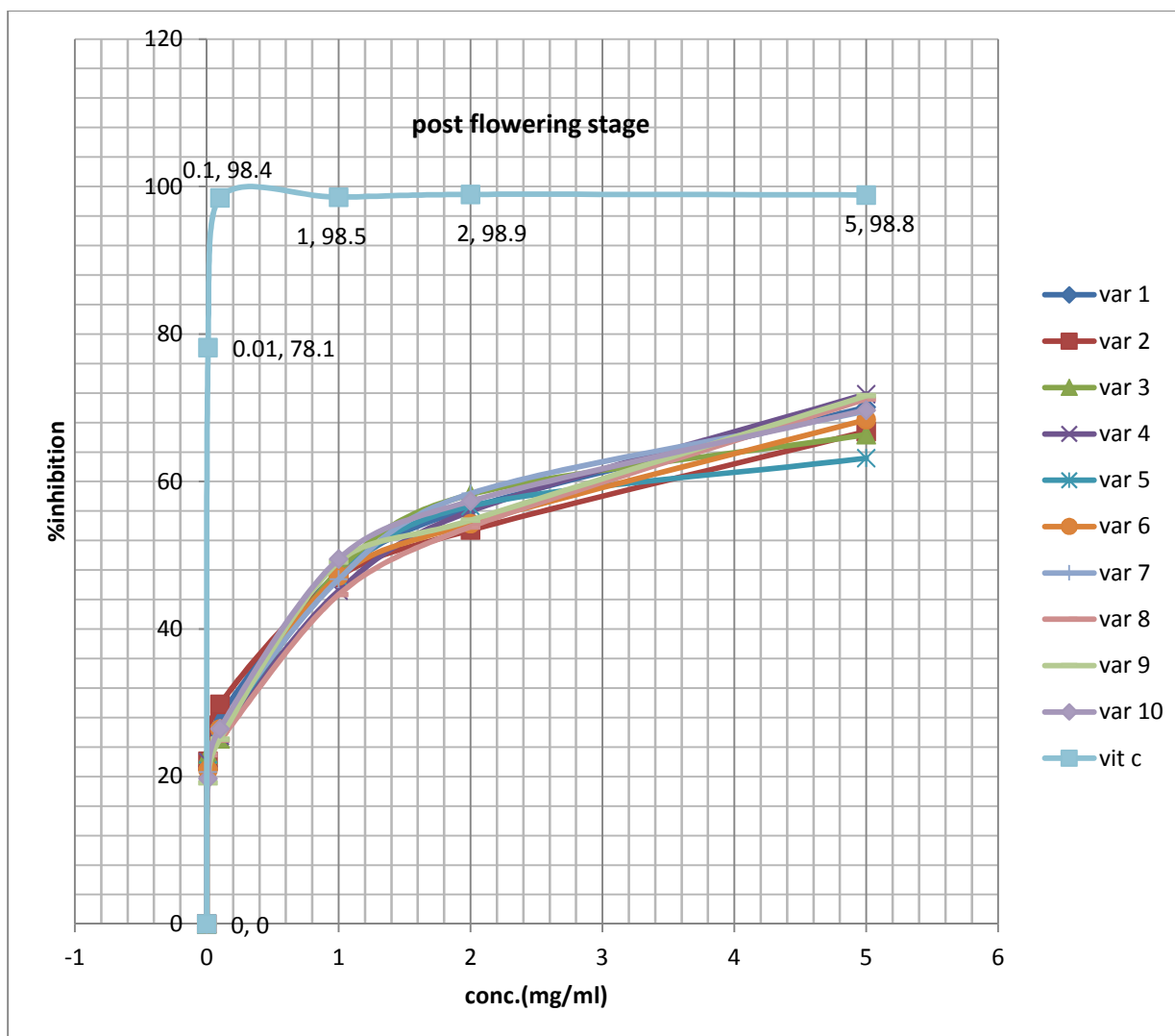


Figure 2: Inhibition (%) of DPPH against concentration of extracts of amaranth leaves harvested at post flowering stage and Vitamin C

The antioxidant activity is contributed to by the various phytochemicals with antioxidant activity. These include vitamin C, carotenoids and flavonoids. However, vitamin C is heat labile, it is also easily dissolved and most of it may have been lost during sample extraction for the antioxidant activity analysis.

4.0 Conclusion

There were significant differences ($p \leq 0.05$) in the phytochemical and anti-nutrient content between the varieties and at the different harvest stages. As the plants matured, the antinutrient content increased. The antinutrient activity also increased with maturity, from vegetative to post flowering stage. The amaranth leaves should be consumed before flowering as they have generally lower anti-nutrients. Selection of the varieties can be done according to their anti-nutrient and phytochemical content

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