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Optimal time for preparing *Amaranthus paniculatus* L. herb

Lala Stepanyan[1*], Tatevik Sargsyan[1,2], Samvel Ghazaryan[1], Monika Israyelyan[1] and Avetis Tsaturyan[1,2]

[1] *Scientific and Production Center "Armbiotechnology" NAS RA, Yerevan 0056, Armenia*
[2] *Yerevan State University, Institute of Pharmacy, Yerevan 0025, Armenia*
**Corresponding author:* lala_stepanyan@rambler.ru

Abstract

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The optimal time for preparing *Amaranthus paniculatus* has been determined based on the dynamics of accumulation of biologically active compounds. The dynamics of accumulation of biologically active compounds and minerals in samples harvested in different phases of vegetation has been studied. The quantitative assessment of elements in leaves of *Amaranthus paniculatus* was carried out using the method of optical emission spectrometry with inductively coupled plasma; the presence of 20 elements was established. The content of 3 macroelements (K, Ca, Mg), 2 mesoelements (Fe, Na) and 14 microelements (Ba, Al, Fe, Mn, Ni, V, Cr, Co, Cu, Zn, Se, Pb, Ag, Tl, Sr, As) was determined. In the series of macroelements, calcium and potassium dominated, in the series of microelements – barium, aluminum, magnesium and zinc. As a result of the primary phytochemical screening, it was found that *Amaranthus paniculatus* was a source of important classes of biologically active substances and minerals. At the same time, it is an environmentally friendly species since it does not accumulate toxic compounds. It has been established that *Amaranthus paniculatus* as a food additive is better to collect in the phase of mass flowering and fruiting.

Keywords: Amaranthus; minerals; vitamin C; phenolics; flavonoids; organic acids

Introduction

Currently, integrated study of many biologically active compounds is still an urgent problem in science and biotechnology. Biologically active compounds are widely used in various important fields such as foodstuffs, agriculture, medicine and veterinary medicine (Paredes-López et al., 2010). Some of them are used in the human diet, others are of medicinal value being a source of vitamins and minerals (Szajdek & Borowska, 2008). The composition of biologically active substances (BAS) depends on the natural characteristics of plants. Useful phytochemicals can be divided into several groups: phenols (flavonoids, tannins, carotenoids), vitamins, organic acids, as well as sulfur and nitrogen – containing compounds such as amino acids, atropines etc. The concentration of BAS in plants depends on the plants variety and species, climatic factors, processes of ripening and timing of harvesting and crop storage. Therefore, it is very important to determine the plant composition at different periods of fruiting in order to determine the optimal harvest time. Considering all this, we chose *Amaranthus paniculatus* as an object for study.

The genus *Amaranthus* L. (family *Amaranthaceae*) contains about 75 species growing in temperate zones of the globe. Since amaranth contains a complex of biologically active compounds, it often used in the pharmaceutical and food industries (Vysochina et al., 2012). Some species of amaranth are used as a crop (Saubhik, 2016). Amaranth is rich in substances of secondary origin, on which its medicinal properties depend. Pharmacological studies have shown that various species of amaranth exhibit anti-inflammatory, hepatoprotective, antipyretic, antidiabetic, spermatogenic, antiproliferative, antifungal and other effects. (Huerta-Ocampo & Barba de la Rosa, 2011; Peter & Gandhi, 2017). Flavonoids, vitamins and other BAS were found in *Amaranth* leaves (Amin et al*.,* 2006). *Amaranth* is known to have a fairly rich elemental composition (Kachiguma et al., 2015). Despite the fact that amaranth herb is actively used in folk medicine, as well as in the composition of dietary supplements, there are no data on the standardization of this type of raw material. When developing standardization criteria, it is necessary to take into account a number of parameters including the content of active substances in the preparation of a medicinal plant material. The need for a detailed study of the dynamics of accumulation of biologically active compounds in different phases of amaranth development is due to the fact that the total content of flavonoids, phenol compounds, vitamins, carboxylic acids varies significantly during preparing raw materials at different stages of vegetation.

It is very important to determine the optimal time for harvesting amaranth grass, depending on the content of biologically active compounds. Besides, the task of studies is to consider the level of elements concentration from the viewpoint of both ecotoxicology, and their importance for the physiological processes in humans and animals (copper, zinc and manganese in small quantities are irreplaceable for living organisms), to identify factors affecting the accumulation of elements in plants.

Materials and Methods

To determine the optimal timing for the harvesting of *Amaranthus paniculatus* herb, the dynamics of accumulation of biologically active compounds and minerals in samples harvested in different phases of vegetation was studied.

Plant material and preparation of extract

The harvesting of *Amaranthus paniculatus* as herb, as a medicinal plant material was carried out in the Kotayk region of the Republic of Armenia in 2022, in the following phases of the plant development:

Ph_1 – budding phase – from the moment of appearance of the first formed buds of inflorescences to their opening (harvest period: May-June 2022);

Ph_2 – mass flowering phase – more than half of the buds of inflorescences are open: (harvest period: August 2022);

Ph_3 – fruiting phase (harvest period: October 2022).

The plant was dried in the shade at room temperature, then crushed into a powder. 150 ml of 30% ethanol was added to the dried plant powder (5 g) in a flat-bottomed flask and extracted at 60°C for 4 h. The extract was filtered, the filtrate was concentrated at a temperature of 50–60°C under reduced pressure. The extract was dried, weighed and stored at 4–5°C for experimental use.

Total content of phenols

The total content of phenols in the extract was determined by Folin-Ciocalteuet method (Kaur & Kapoor, 2002). 200 µl of the crude extract (1 mg ml^{-1}) was diluted with distilled water to 3 ml, 1 ml of 10% Folin-Ciocalteu reagent was added, after which 2 ml of 10% sodium carbonate solution was added. The mixture was left for another 20 min in the dark. Optical density was measured at 730 nm. The total phenol content was calculated from the calibration curve and the result was expressed in milligrams of gallic acid equivalents per gram dry weight.

Total content of flavonoids

The total content of flavonoids in the crude extract was determined by the colorimetric method (Chang et al*.,* 2002). 50 µl of crude extract (1 mg ml^{-1}) was adjusted to 1 ml with ethanol, then the volume of the mixture was adjusted to 4 ml of distilled water, and then 0.5 ml of a 5% $NaNO_2$ solution was added. After a 10-minute incubation, 0.5 ml of 10% $AlCl_3$ solution was added. The mixture was kept for 10 min, then 2 ml of 1 N NaOH solution was added, and the final volume of the mixture was adjusted to 10 ml with distilled water. The mixture was left for 40 min. Optical density was measured at 510 nm. The total content of flavonoid was calculated from the calibration curve and the result was expressed as milligrams of rutin equivalent per gram of dry weight.

Determination of vitamin C

The amount of vitamin C was determined by the iodometry with some modifications (Spiridonova, 2016). 25 ml of a 2% hydrochloric acid solution was added to 1 g of dried, ground raw material (hydrochloric acid prevents the process of oxidation of ascorbic acid), crushed for 5 min, filtered, 25 m of hydrochloric acid was added again to the raw material and the process was repeated 4 times. Hydrochloric acid solutions were filtered into a 100 ml measuring cylinder, in the end the volume was adjusted to the mark, a starch indicator was added to the obtained solution and titrated with 0.125% iodine solution (1 ml of 0.125% iodine solution is equivalent to 0.877 mg of ascorbic acid).

Extraction and titrimetric determination of free organic acids

The total amount of free organic acids in the extract was determined by the titrimetric method with modifications

(Maslov et al., 2021). 1.0 g of the crushed raw material was placed in a flat-bottomed flask (250 ml), 200 ml of boiled water was poured and kept in a boiling water bath for 2 h. The extract was cooled. After cooling the volume of the solution was adjusted to 250 ml (solution A). Indicators (5 drops of 0.1% methylene blue solution, 5 drops of 1% alcohol solution of phenolphthalein) and 100 ml of freshly boiled water were added to 10 ml of solution A. The solution was titrated with 0.1N sodium hydroxide solution. In parallel, a control experiment was carried out without adding the extract.

$$X = \frac{(V_0 - V_k) \cdot 0.0067 \cdot 250 \cdot 100 \cdot 100}{10 \cdot m \cdot (100 - W),}$$

where:

X – amount of free organic acids, %;

V_0 – is the volume of NaOH (0.1 N) used for titration, ml;

V_k – is the volume of NaOH (0.1 N), spent for titration in a blank experiment, ml;

m – is the mass of raw material, g;

W – moisture of raw material, %;

0.0067 – is the amount of maleic acid, equivalent to 1 ml of sodium hydroxide solution (0.1 N), g.

Determination of macro and microelements

Quantitative determination of elements was carried out by optical emission spectrometry with inductively coupled plasma (Agilent 5800 VDV ICP-OES). For the quantitative determination of elements, first, sample preparation is carried out. Sample preparation was carried out in an acidic medium under heating conditions by decomposition using a microwave oven (Anton Paar – Multiwave GO PLAS) (Khavezov & Tsalev, 1983). Dry plant material was weighed in a microwave dish (0.3075) and 5 ml of concentrated (69%) HNO_3 was added to it. Decomposition was carried out in a microwave oven at 180°C for 10 min, after which the amount of the resulting solution was brought to 50 ml with deionized water and analyzed. For the quantitative determination of elements, the analysis was carried out on an ICP-OES device using the latest standards (multistandards) in different dilutions, with detection (µg/g).

Results and Discussion

To determine the changes in the amount of the main classes of extractive substances, such important chemical components as the total amount of vitamin C, phenolic compounds, flavonoids and free organic acids of *Amaranthus paniculatus* were investigated.

Vitamin C is an important component for the activity of living organisms. Vitamin C is a natural antioxidant, which is necessary for the biosynthesis of collagen and carnitine. Vitamin C deficiency causes scurvy (Grosso et al., 2013).

Studies showed that the content of vitamin C is highest at the mass flowering stage at 192.15 mg/100 g (Table 1).

Table 1. Total vitamin C content[a] of *A. paniculatus,* different phases of flowering

Budding phase (Ph_1)	166.15±2.5
Mass flowering phase (Ph_2)	192.15±3
Fruiting phase (Ph_3)	178.26±2.7

[a] mg in 100g

According to the U.S. Food and Drug Management (FDA USA, 2013), the daily dose of vitamin C is 60 mg. As studies have shown *Amaranthus paniculatus* can be a source of vitamin C, especially if the plant is used in the flowering phase.

The level of phenols content is a very important indicator. The total content of phenols in ethanol extracts was respectively Ph_1 – 45.2±2, Ph_2 – 58.5±1.5, Ph_3 – 52.3±2 eq. of gallic acid/g, and the total content of flavonoids Ph_1 – 23.6±2, Ph_2 – 31.5±1.5, Ph_3 – 27.3±2 eq. of rutin/g (Table 2). Phenolic compounds (flavonoids, including flavones, flavanols and condensed tannins) have redox properties. Since the hydroxyl groups of phenols provide their ability to scavenge free radicals, the total concentration of phenols can be used to screen for antioxidant activity. Plant flavonoids have antioxidant activity in vitro and in vivo conditions (Soobrattee et al., 2005; Shimoi et al., 1996; Geetha et al., 2003).

Early investigations have also shown that *A. paniculatus* leaves contain sufficient amounts of phenols, flavonoids and ascorbic acid. Polyphenols, ascorbates and flavonoids are known as potential antioxidants and free radical scavengers. Research showed that *A. paniculatus* extracts were rich in antioxidant phytochemicals, suggesting that the leaves may be used as an important source of natural antioxidants with health protective potential (Sreelatha et al., 2012; Gins et al., 2021).

Free organic acids were determined. The research has shown that organic acids accumulate in the fruiting phase.

Table 2. Total content of phenolics and flavonoids in ethanolic extract of *A. paniculatus*

	Ph_1	Ph_2	Ph_3
Total phenolics content[a]	45.2±2	58.5±1.5	52.3±2
Total flavonoids content[b]	23.6±2	31.5±1.5	27.3±2

[a]mg gallic acid equivalent/g DW

[b]mg rutin equivalent/g DW

The total content of organic acids in the extracts was Ph_1 – 5.15±1.5%, Ph_2 – 5.54±1.65%, Ph_3 – 7.03±1.7%, respectively. Organic acids have a wide range of biological activity. They have a bile-expelling effect, regulate the secretion of bile, have bactericidal properties, and normalize the activity of the digestive system.

Macro and microelements were also determined. On the one hand, minerals as components of enzymes play an important role in the human body. On the other hand, their increase can cause adverse health effects such as poor fertility, endocrine disruption, and cancer-related genotoxicity (Bateman et al., 2018; Rahman et al., 2012; Renieri et al., 2017; Al-Hossainy et al., 2017). In addition, it is important to note that lead accumulates in the human body, causing bad consequences: heavy metal poisoning (Collin et al., 2022). The study showed that the content of metals in all stages of plant development was within the normal range and comparable to commercial vegetables. The presence of 20 elements at different stages of life of *Amaranthus panucalutus* was established (Table 3). The amount of such microelements as zinc, manganese, chromium, barium is greater in the budding phase (Ph_1), iron, aluminum and lead accumulate in the flowering phase (Ph_2), and the amount of macroelements – calcium, magnesium, potassium, and mesoelement – sodium, and microelements – thallium, selenium, nickel, strontium and copper predominate in the fruiting phase (Ph_3). Heavy metals and arsenic do not accumulate in the studied plant either (Table 3) and their quantitative content is within the limits established by the State Pharmacopoeia of the Russian Federation (SP RF) of the 14th edition. As the study has shown

Table 3. The mineral composition of *Amaranthus panucalutus* at different stages of flowering

No.	Element	Wavelength nm	Ph_1 (concentration mg/kg)	Ph_2 (concentration mg/kg)	Ph_3 (concentration mg/kg)
Macronutrients (more than 1000 µg/g)					
1	K	766.491	25968.97	25551.15	26009.31
2	Ca	422.673	21143.89	19353.86	22777.98
3	Mg	280.270	12460.44	11513.26	14976.71
Mesoelements (100–1000 $\mu g\ g^{-1}$)					
4	Na	589.592	280.54	201.32	293.64
5	Fe	259.940	393.86	455.94	420.19
Microelements (0.01–100 µg/g)					
6	Ba	455.403	83.06	67.5	78.86
7	Al	396.152	61.07	64.21	53.23
8	Mn	257.610	25.94	22.86	25.88
9	Zn	163.18	20.75	15.24	20.70
10	Sr	407.771	16.03	13.95	18.34
11	Cu	324.754	8.38	10.70	12.94
12	Cr	267.716	4.38	3.62	3.76
13	Ni	231.604	3.13	3.85	4.08
14	Se	196.026	2.51	3.34	3.65
15	Co	230.786	0.34	0.35	0.31
16	V	292,401	0.32	0.31	0.33
17	As	188.980	0.223	0.205	0.212
18	Pb	220.353	0.178	0.198	0.182
19	Ag	328.068	0.045	0.048	0.045
20	Tl	190.794	0.00175	0.00184	0.00179
21	Be	313.042	0.00	0.00	0.00
22	Sb	206.834	0.00	0.00	0.00
23	Cd	214.439	0.00	0.00	0. 00

the plant does not contain cadmium, stibium and beryllium; silver, cobalt and vanadium are contained in small quantities.

These mineral elements play an important role in human life. Thus, for example, Na and K are involved in the ion balance in the human body, provide tissue excitability. Na plays an important role in the transport of metabolites, K has a diuretic effect (Chen et al., 2005). Ca is necessary for normal functioning and regulation of cell permeability (Singh et al., 2020). Ca is the main component of bones and contributes to teeth development (Naumova & Šachl, 2020). Mg is an essential element because it is a cofactor for many enzymes (Chaudhary et al., 2009). Cu plays an important role in protein synthesis (Qian et al., 2007). Ni is also an essential element and its daily consumption is recommended for good health (Sevin et al., 2023). The Zn concentration was higher in Ph_2. Zn is a immune response stimulator, membrane stabilizer. Its deficiency leads to growth retardation (Frydrych et al., 2023). The content of Mg was higher in the Ph_1 and Ph_3. Mg is very essential for the formation of hemoglobin (Zoroddu et al., 2019). The Fe concentration was higher in Ph_2 Cd, As, Be and Pb are best known for their toxicological properties (Rahman & Singh, 2019). Low levels of Pb, As and the absence of Cd and Be in *Amaranthus panucalutus* indicate its non-toxicity.

Mineral elements play an important role but when used they should be combined with various biologically active compounds – vitamins, amino acids, etc. *A. panucalutus* has the similar balanced combination.

Conclusion

Thus, as a result of the primary phytochemical screening it has been established that *Amaranthus panucalutus* is a source of important classes of BAS, such as vitamins, organic acids, flavonoids, salts of calcium, magnesium and others. It has also been detected that it is better to collect – *A. panucalutus* as a food additive during phases of mass flowering and fruiting. At the same time, *A. panucalutus* is an environmentally friendly species, since it does not accumulate toxic compounds.

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