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Research Article

Determination by UHPLC – UV – MS of polyphenol content of *Amaranthus retroflexus*

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Abstract

Having a very large area globally, the genus *Amaranthus* has been known since ancient times and used as a medicinal plant used in the treatment of various ailments. For the treatment of gastrointestinal disorders, the main constituents considered active are compounds from the polyphenol class. In order to identify and quantify some of these compounds, this paper briefly presents a UHPLC–UV–MS analysis study of polyphenolic compounds from *Amaranthus retroflexus*. Through our study we identified a high concentration of rutin. Thus, if rutin is intended to be used in medical practice, only the leaves will be used, the rest of the plant would produce an unwanted dilution. In addition, $C_{16}H_{18}O_9$, $C_9H_8O_4$, $C_7H_6O_4$, $C_9H_8O_3$ and $C_{10}H_{10}O_4$ were identified. It has been observed that the leaves contain flavonoids in a higher amount than other parts of the plant. Through our study, we contributed to establishing the working parameters necessary to perform the analyses. For the first time, we have indicated the organs with the highest content of flavonoids, from the composition of the plants in the Oltenia area, Romania. For medical practice, the results obtained by us can represent important milestones in the production of pharmaceutical preparations.

Keywords: *Amaranthus retroflexus*; gastrointestinal disorders; flavonoids; UHPLC–UV–MS

Introduction

Amaranthus retroflexus, is a plant that forms bushes (Pammel, 1903), and is included in the list of species harmful to agricultural crops (Ciocârlan *et al.*, 2004; Saravanan *et al.*, 2013). Originally from the tropical Americas, the plant was then brought and acclimatized on most continents over fairly large areas. Its height can reach almost 3 m. The leaves are lance-shaped and 10-15 cm long. Both male and female flowers can be found on the same stem. The pollen of *A. retroflexus* may be a cause of IgE-mediated respiratory allergies, especially in

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semi-desert countries such as Iran, Kuwait, Saudi Arabia (Socea *et al.*, 2015; Tehrani *et al.*, 2010; Torres *et al.*, 1997). The fruit is a capsule less than 2 mm long (Elias *et al.*, 2009) containing a tiny black seed. Another name for *Amaranthus retroflexus* is "pigweed" because the plant grows on land where pigs are fed (***). *A. retroflexus* can be a toxic plant to cattle, causing extensive degeneration and necrosis of proximal and distal tubules with interstitial fibrosis and tubular proteinosis (Casteel *et al.*, 1994; Kerr *et al.*, 1998; Tehrani *et al.*, 2010).

In some specialized works, the chemical composition of Amaranth was determined and it was discovered that it has a high content of calcium, ascorbic acid, iron and many precursors of vitamin A. Amaranth culture is not an expensive culture, which is why it is a vegetable cheap. From this plant, in general, the stems and leaves are consumed, which have an increased content of ascorbic acid and proteins very necessary for human nutrition (Kongdangun *et al.*, 2021).

The Amaranthus genus has a very extensive geographic range and has been used as a medicinal plant from ancient times to treat a variety of diseases (Kongdangun *et al*., 2021). Considering that the species of the genus *Amaranthus* have been investigated for a variety of potential pharmacological benefits, such as hepatoprotective, cardioprotective or antidiabetic effects (Würtzen *et al.*, 1995; Fox *et al.*, 2011; Robu *et al.*, 2016; Zeashan *et al.*, 2008) the species *A. retroflexus* has been very little investigated what which leaves us room to investigate the polyphenol content by UHPLC - UV – MS. In a paper presented at the ICNFS 2013, Lolita Tomsone and Co. presented a study on the freezing preservation methods of amaranth products and the influence of freezing on the chemical composition (Tomsone *et al.*, 2013). Compounding of the flora in the region of Serbia, Terekhina *et al*. (2021) discovered entire areas where the *Amaranthus* species was predominant (76 - 100 %). Other groups of researchers have been concerned with the study of pigments and nutrients in the leaves of Danta (*Amaranthus lividus*) (Sarker *et al.*, 2022). The diversity of the composition of the *Amaranthus* species posed the question of the possibility of toxicity. This has been studied by various universities, with interesting results mentioned in the literature (Dinu *et al.*, 2017; Amoli *et al.*, 2009; Chirigiu *et al.*, 2012). From the specialized literature (Escudero *et al.*, 1999), the moisture content, ether extract and ash were determined by the AOAC methods (AOAC, 1990).

From the specialized literature you can find out the moisture content, the ether extract and the ash were determined by the AOAC methods (AOAC, 1990), the metal content Zn, Fe, Cu, Na, K and Mn which was determined by atomic absorption spectrophotometry (Escudero *et al.*, 1999), phosphorus and calcium were determined colorimetrically (Stuffins, 1967; Welcher, 1966), crude protein, N × 6.25, was determined by the Kjeldahl method, as modified by Winkler (Jacobs ,1973), soluble and insoluble fiber contents were determined according to Prosky *et al.* (1988), and fatty acids were extracted according to Stanbie *et al.* (1976) and determined as methyl esters by gas chromatography (EPA , 1980). In our study, we managed to determine by UHPLC – UV – MS a series of polyphenolic compounds from *Amaranthus*.

Materials and Methods

Sample preparation

The plant product obtained from *Amaranthus retroflexus* collected from several places of spontaneous flora in Oltenia, a region in the southwest of Romania, was pulverized and sieved through a 160/350 μm sieve. One gram of the obtained plant product was extracted with 10 mL methanol 70%. Initially, the plant product together with the solvent was left under continuous stirring for 30 minutes. Secondly the mixture was ultrasounded for 15 minutes and centrifuged at 10000 RPM for another 15 minutes. The obtained supernatant was filtered using 0.2 μm syringe filters and transferred to autosampler vials to be used as such.

Test preparation

The solution obtained by dissolving 8 mg of each reference compound in 100 mL methanol. The stock solution was kept refrigerated at 4 °C until use. The calibration curve concentrations were obtained by diluting the previous mentioned stock solution.

UHPLC-UV-MS method

The separation of polyphenols was carried out on a Waters (Milford, Massachusetts, USA) Arc System coupled with a Waters 2998 PDA detector and a Waters QDa mass detector. The column used was a Waters CORTECS C18 (4.6 × 50 mm, 2.7 μm) eluted with solvent A (0.1% formic acid in **H_2O**), solvent B (0.1% formic acid in CH_3OH) and solvent C (0.1% formic acid in **C_2H_3N**). Solvent B was set at 1% during the entire separation. The gradient was as follows: 0-4 min 3%-14% C, 4-9 min 14% to 39% C, 9-11 min 29% to 3% C. The flow rate of the mobile phase was set at 1.0 mL/ min. The temperature was maintained at 35 °C. A volume of 5 μL was injected. All samples were kept at 20 °C during the entire analysis.

The chromatogram was obtained using the 2998 PDA detector at 280 nm. Compounds analyzed using QDa mass detector with electrospray ionization (ESI) source. Capillary tension was stabilized at 0.8 kV, cone voltage was kept at 20 V and the mass spectra were recorded in negative ion mode at interval 100–800 m/z. Quantification was established in SIR mode for each compound (as shown in Table 1) using external calibration curves prepared for each standard. The equipment was controlled using the EmPower 3 software package.

Results

Experimental data on the UHPLC – UV – MS analysis of polyphenolic compounds from *Amaranthus retroflexus* are presented as follows:

Table 1. Linearity range and detection characteristics of the reference polyphenolic compounds

Compound name	Main ion [*m/z*]	Retention time t_R [min]	Linearity range [ng]	R^2
Protocatechuic acid	153	1.639	0.5–5	0.998
Chlorogenic acid	353	3.063	100–400	0.998
Caffeic acid	179	3.306	0.5–5	0.999
p-Coumaric acid	163	4.435	0.5–5	0.999
Ferulic acid	193	5.130	1–10	0.998
Rutin	609	5.561	0.5–5	0.998
Quercetin	301	7.528	1–10	0.994

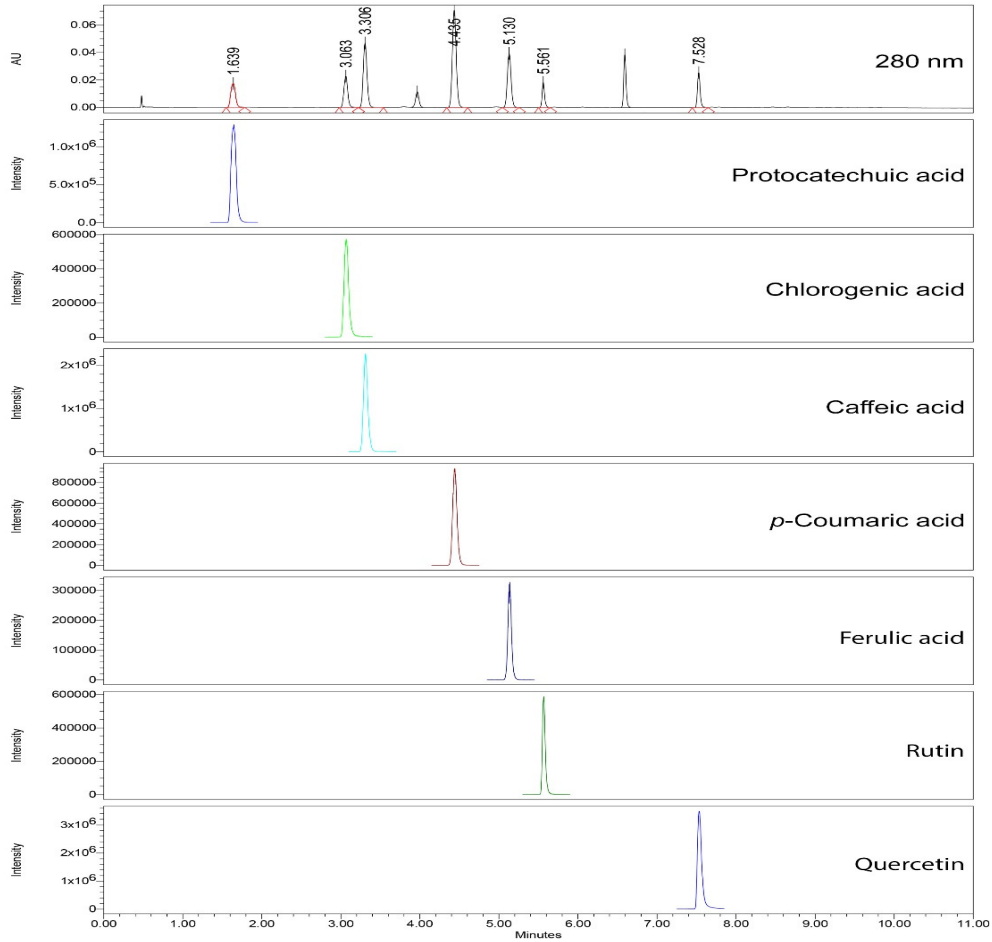

Figure 1. UHPLC chromatograms obtained on the basis of 280 nm wavelength and selective ion recording mass spectra (MS – SIR) for the reference polyphenolic compounds

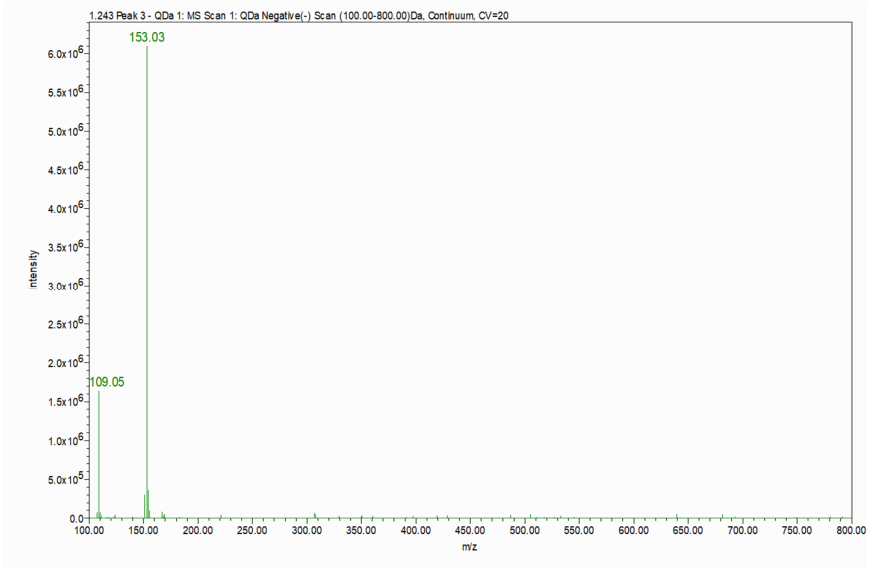

Figure 2. Mass spectrum of the protocatechuic acid (m/z 153)

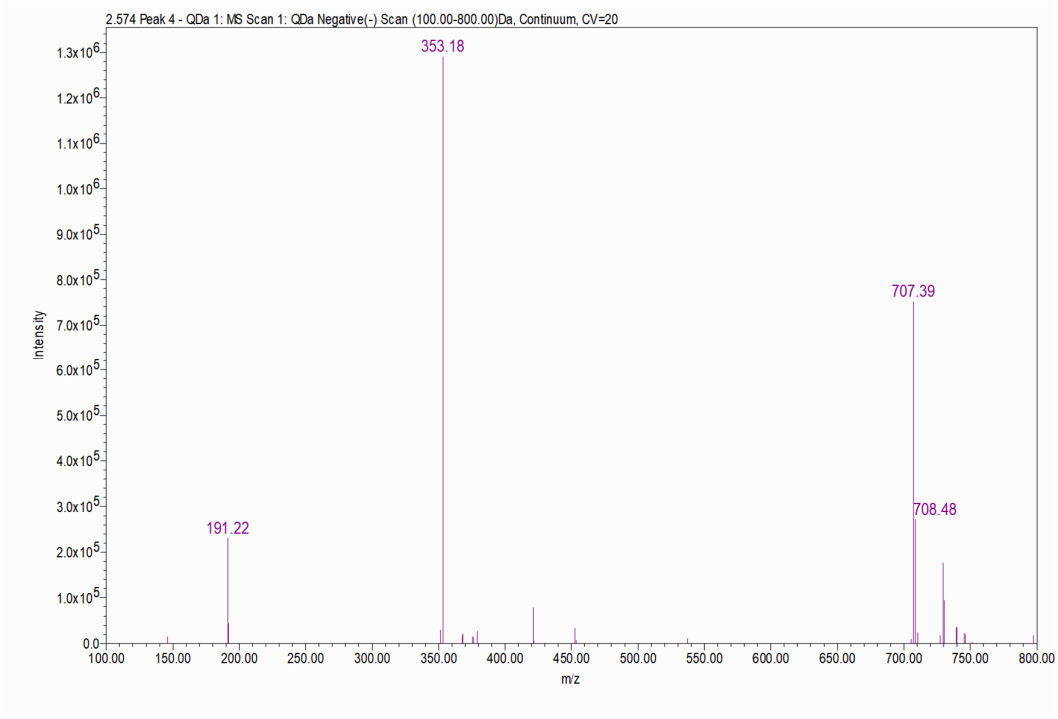

Figure 3. Mass spectrum of the chlorogenic acid (m/z 353)

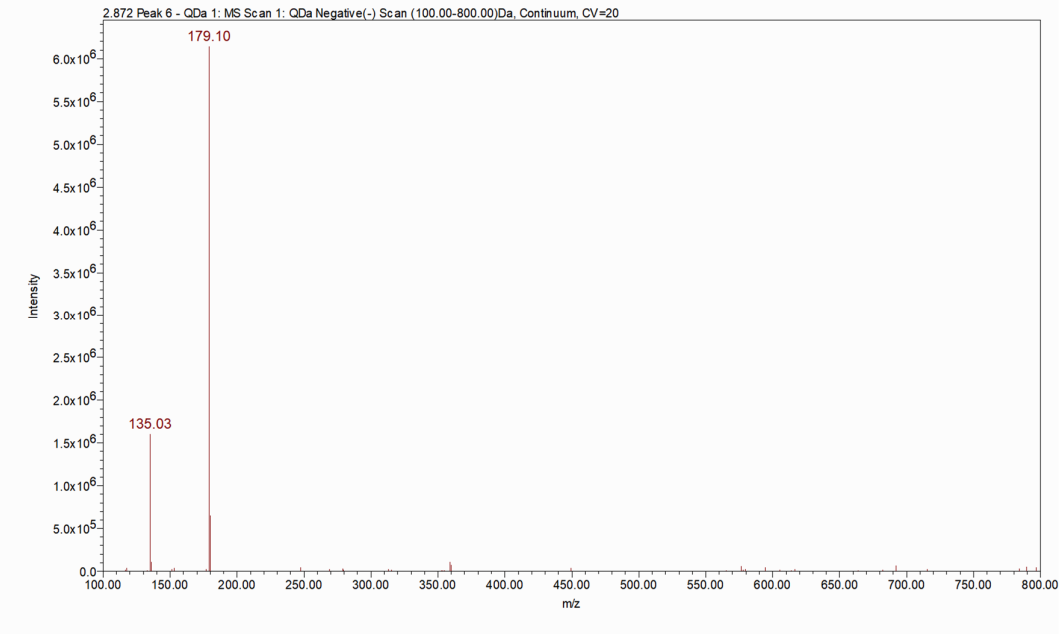

Figure 4. Mass spectrum of the caffeic acid (m/z 179)

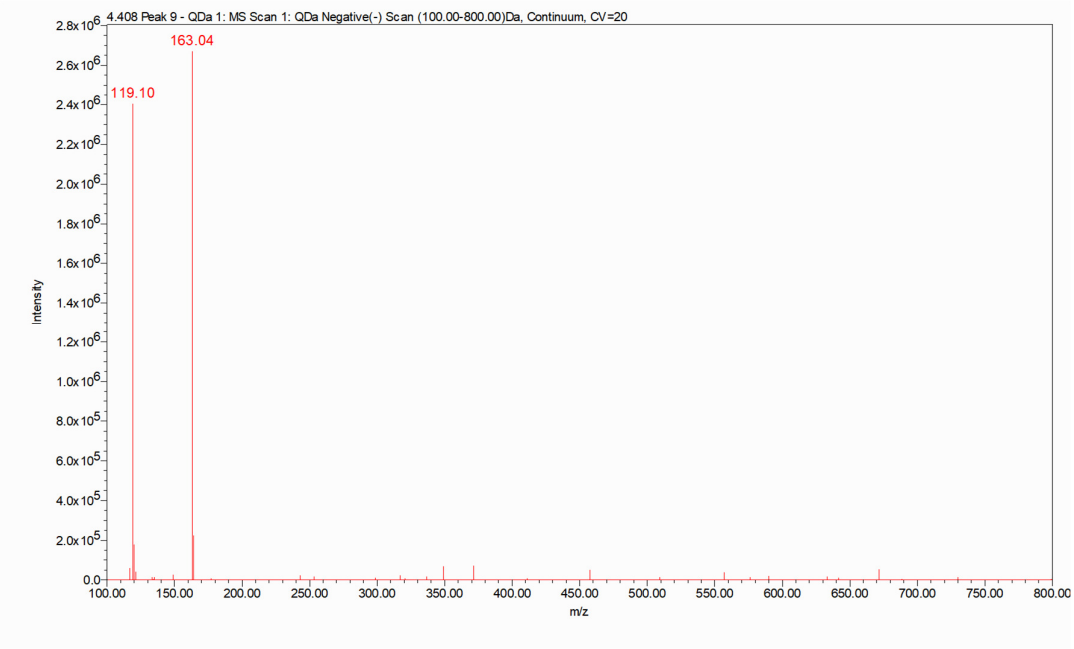

Figure 5. Mass spectrum of the *p*-coumaric acid (m/z 163)

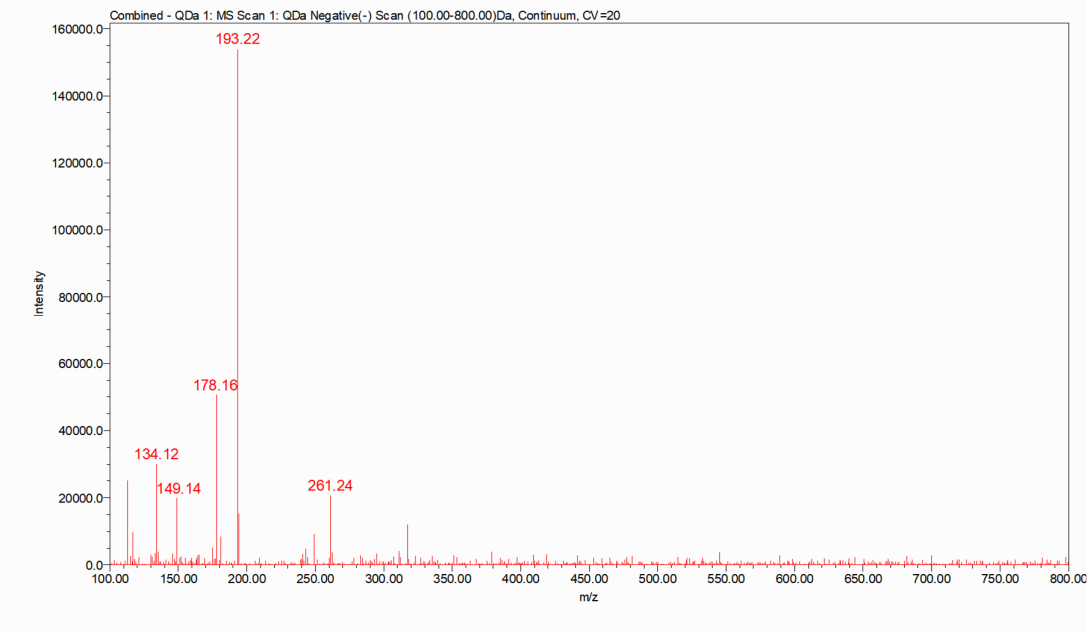

Figure 6. Mass spectrum of the ferulic acid (m/z 193)

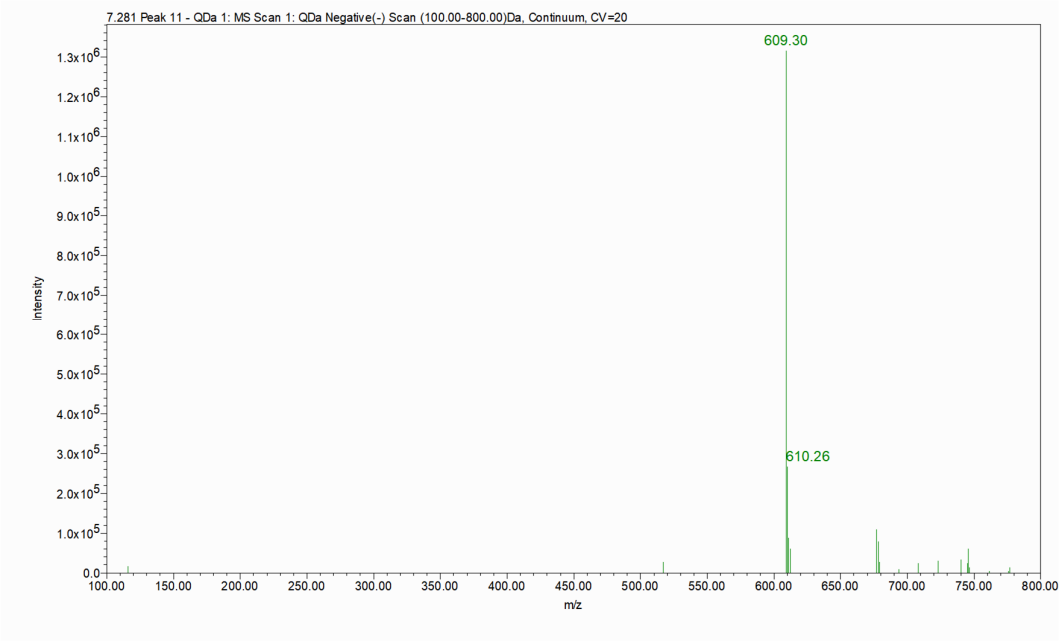

Figure 7. Mass spectrum of the rutin (m/z 609)

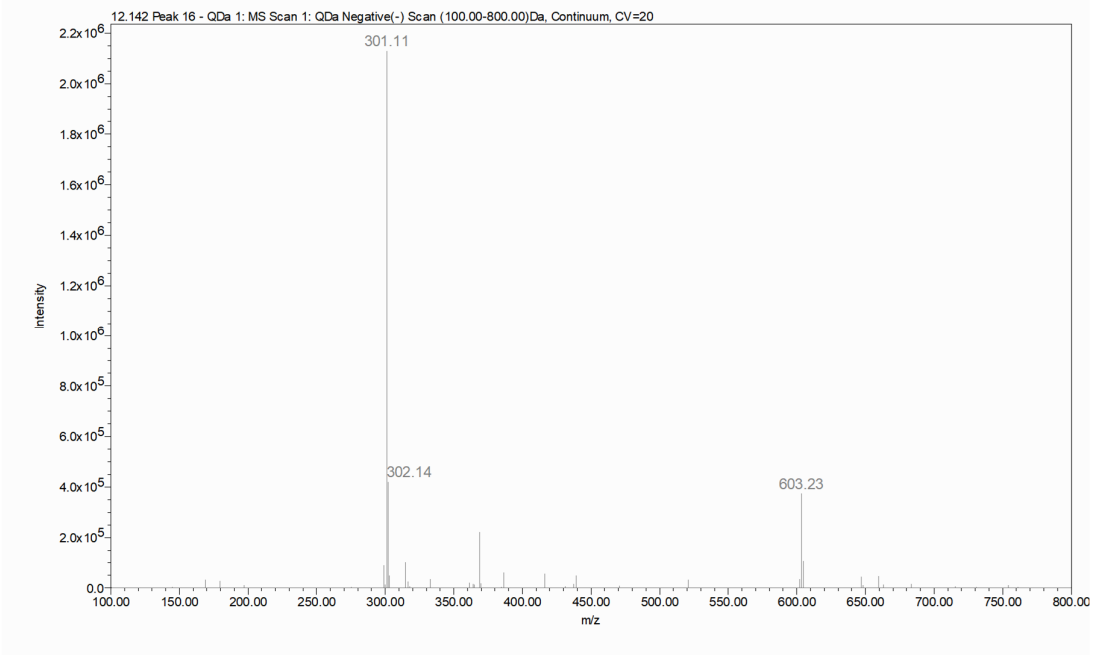

Figure 8. Mass spectrum of the quercetin (m/z 301)

Experimental results obtained through UHPLC – UV – MS analyses performed on the plants of the species Amaranthus retroflexus

Amaranthi folium

Results are presented in Figures 9-11; Table 2.

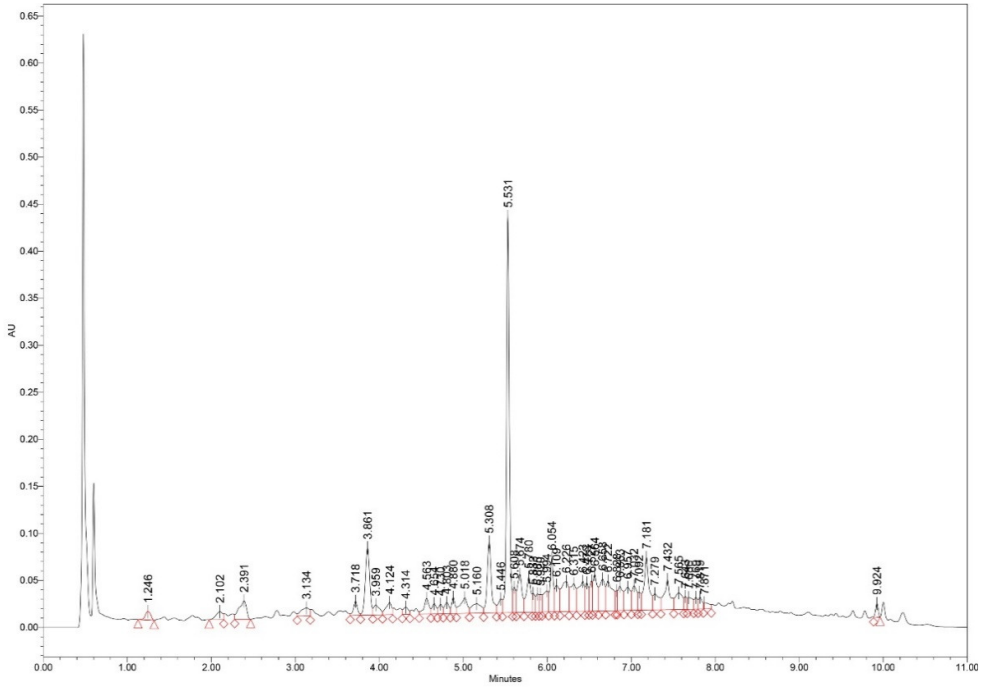

Figure 9. UV (280 nm) chromatogram of the *Amaranthi folium* extract

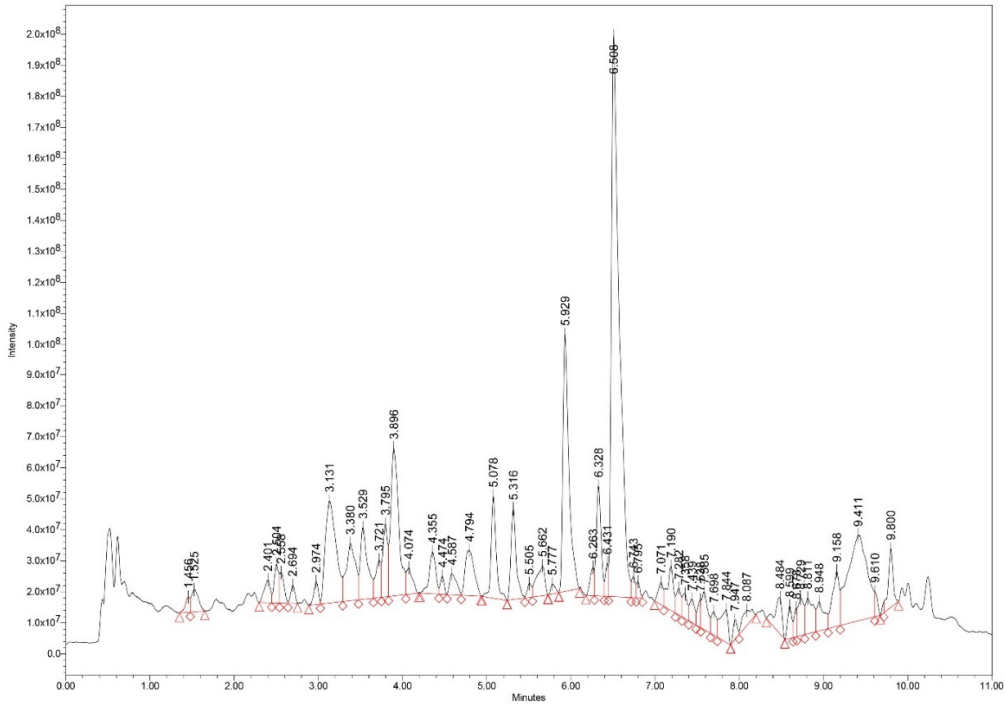

Figure 10. MS-TIC chromatogram of the *Amaranthi folium* extract

Table 2. Concentration of polyphenolic compounds in the hydroalcoholic extract (prepared in 70% methanol) and in the medicinal plant product *Amaranthi folium*

Compound name	Concentration in hydroalcoholic extract (methanol 70%) [ng/µl]	Concentration in natural product [µg/g]
Protocatechuic acid	0.610	6.10
Chlorogenic acid	1.583	15.83
Caffeic acid	0.362	3.62
p-Coumaric acid	0.405	4.05
Ferulic acid	1.890	18.90
Rutin	33.225	332.25
Quercetin	0.056	0.56

Amaranthi stipes

The results are presented in Figures 11-12 and Table 3.

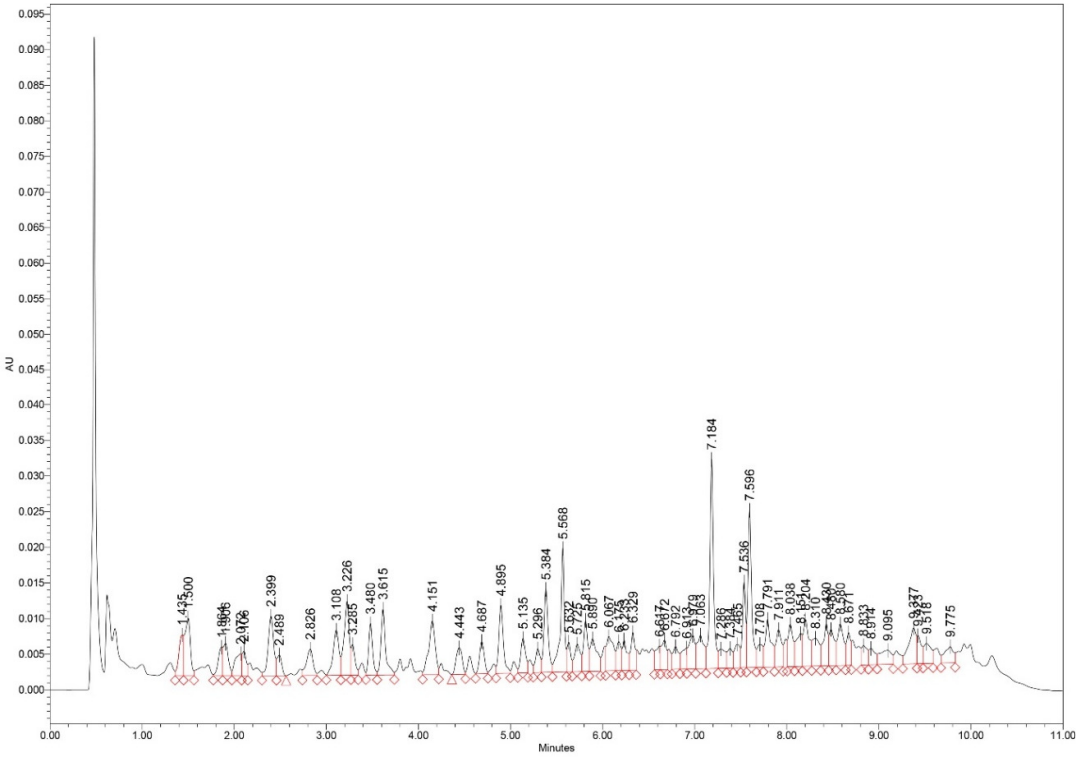

Figure 11. UV (280 nm) chromatogram of the *Amaranthi stipes* extract

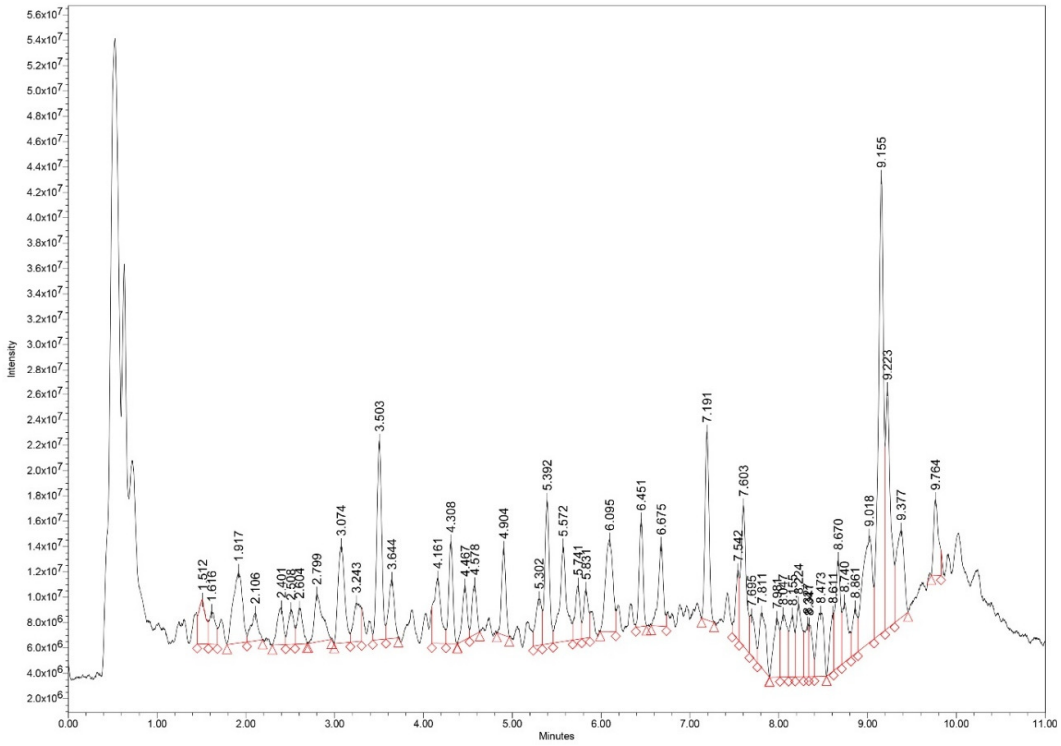

Figure 12. MS-TIC chromatogram of the *Amaranthi stipes* extract

Table 3. Concentration of polyphenolic compounds in the hydroalcoholic extract (prepared in 70% methanol) and in the medicinal plant product *Amaranthi stipes*

Compound name	Concentration in hydroalcoholic extract (methanol 70%) [ng/µl]	Concentration in natural product [µg/g]
Protocatechuic acid	0.116	1.16
Chlorogenic acid	-	-
Caffeic acid	-	-
p-Coumaric acid	0.437	4.37
Ferulic acid	1.429	14.29
Rutin	10.646	106.46
Quercetin	-	-

Amaranthi semen

The results are presented in Figures 13-14 and Table 4.

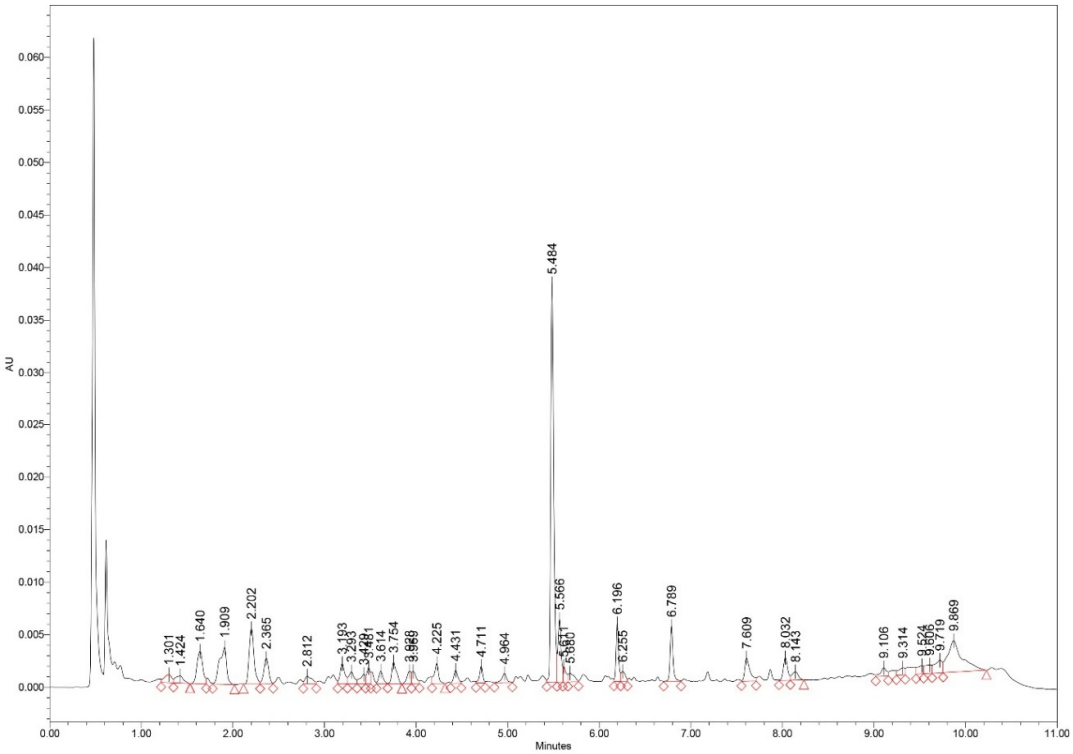

Figure 13. UV (280 nm) chromatogram of the *Amaranthi semen* extract

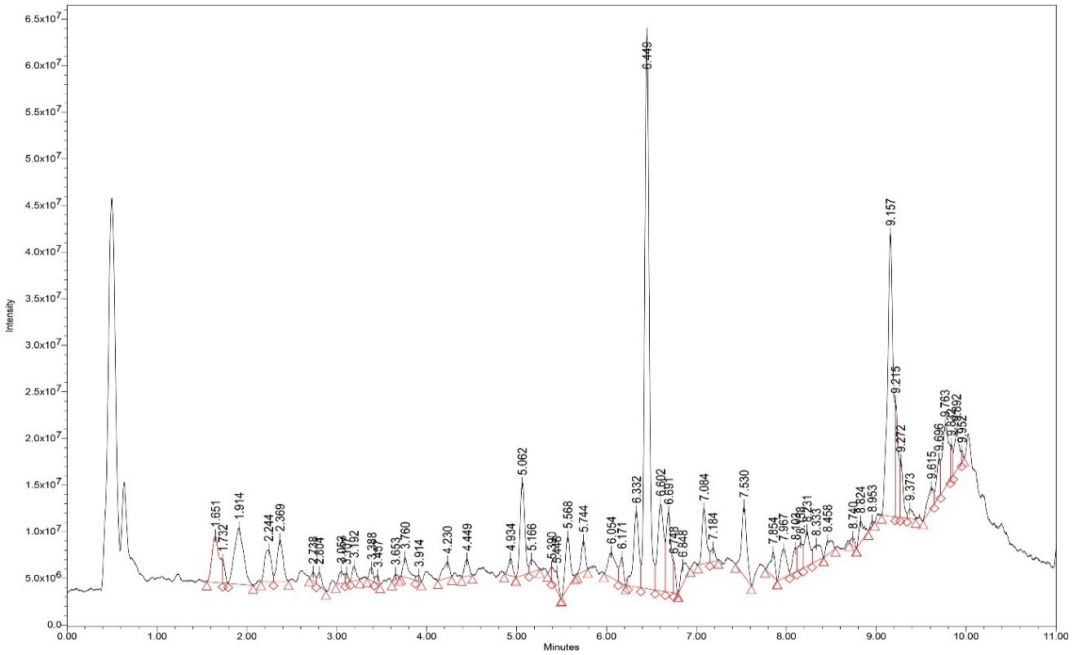

Figure 14. MS-TIC chromatogram of the *Amaranthi semen* extract

Table 4. Concentration of polyphenolic compounds in the hydroalcoholic extract (prepared in 70% methanol) and in the medicinal plant product *Amaranthi semen*

Compound name	Concentration in hydroalcoholic extract (methanol 70%) [ng/µl]	Concentration in natural product [µg/g]
Protocatechuic acid	1.637	16.37
Chlorogenic acid	-	-
Caffeic acid	-	-
p-Coumaric acid	0.032	0.32
Ferulic acid	0.657	6.57
Rutin	4.124	41.24
Quercetin	-	-

Amaranthus retroflexus

Results are presented in Table 5 and Figure 15.

Table 5. Botanical data for *Amaranthus retroflexus*

***Amaranthus* species (Accepted names)**	**Synonyms**
Amaranthus retroflexus L. Weed amaranths (redroot pigweed)	*Amaranthus bulgaricus* Kov.
	Amaranthus curvifolius Spreng.
	Amaranthus delilei Richt. & Loret
	Amaranthus johnstonii Kov.
	Amaranthus recurvatus Desf.

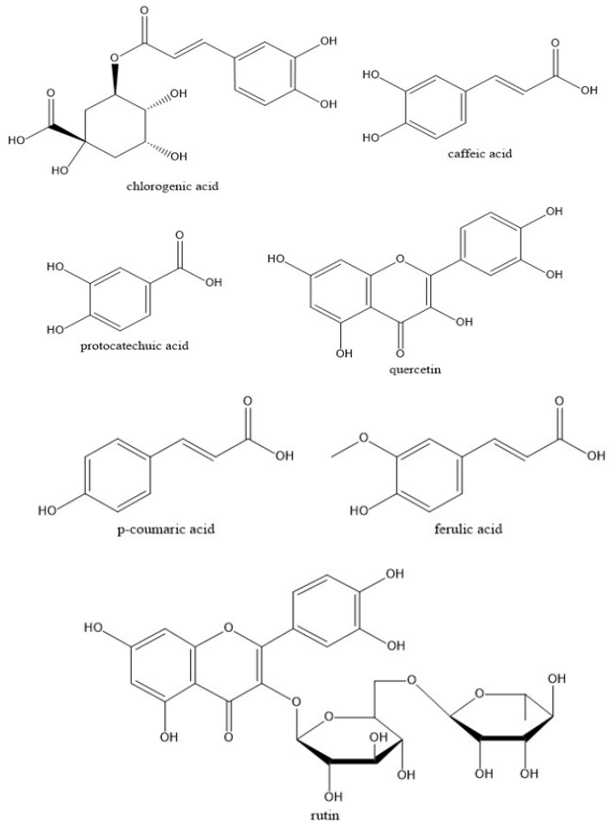

Figure 15. Structures representing all the identified polyphenols in *Amaranthus retroflexus*

Discussion

General issues

Our studies were carried out on 70% alcoholic extracts specific to medical practice, aiming to determine the composition in medically bioactive compounds. The alcoholic extracts of the leaves, stems and seeds of *Amaranthus retroflexus* L were thus successfully analysed. The obtained results can be used in medical practice.

The polyphenols detected in the analysed *A. retroflexus* samples are presented in Tables 2, and 4. Studies have reported that the leaves of *A. retroflexus* are a great source of rutin (332.25 μg/g concentration in natural product *Amaranthi folium*). Our study supports this information and moreover we have identified a high concentration of rutin in the stems as well. We have also identified protocatechuic acid (1 - 16 μg/g concentration in natural product *A. retroflexus*), chlorogenic acid (15,83 μg/g concentration in natural product *Amaranthi folium*), caffeic acid (3,62 μg/g concentration in natural product *Amaranthi folium*), p-coumaric acid (0,3 – 4,3 μg/g concentration in natural product *A. retroflexus*) and ferulic acid (6 – 19 μg/g concentration in natural product *A. retroflexus*). Quercetin and its derivatives are not the major constituents of this species, as seen from the assay (very low concentrations). It is observed that the leaves contain flavonoids in a higher amount than the seeds.

The antioxidant activities of phenolic compounds have been studied by eliminating radicals, slowing the process of lipid oxidation and hydroperoxide formation, not the major constituents of this species, as seen from the assay (very low concentrations). Ferulic, caffeic, chlorogenic, p-coumaric and protocatechuic acids contributed to the antioxidant potential of various natural products. Protocatechuic acid and rutin have inhibitory activity against *Pseudomonas aeruginosa*. Protocatechuic acid sulfamethoxazole combination showed a synergistic mode of interaction. In the nutrient environment, the combinations of gallic and protocatechuic, gallic and caffeic, rutin and quercetin were the best antibacterial agents with synergistic effects. these combinations were selected to test their activity in a meat model system.

Conclusions

In conclusion, this study was carried out on plants harvested from the Oltenia area. It is known that the chemical composition of plants can be influenced by the chemical properties of the soil and climatic factors. For medical practice, the results obtained by us can represent important milestones in the production of pharmaceutical preparations.

From another point of view, the different chemical composition for the different organs of the plants provides indications regarding their selective use in the intended treatments. Thus, if rutin is intended to be used in medical practice, only the leaves will be used, the rest of the plant would produce an unwanted dilution.

The working conditions established by us will be able to be used by other researchers in this field.

Authors' Contributions

Conceptualization: APS and SET; Data organization and analysis: AB, LMEC and IAS; Preparing the samples and conducting the experiment: APS and LMEC; Editing- original draft: IAS and EC; Writing-review and editing: APS; Supervision: AB. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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