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STUDIES ON THE DETERMINATION OF IC50 VALUES OF ETHANOLIC AND METHANOLIC EXTRACTS FROM THE SPECIES *Amaranthus retroflexus* L (Amarantacae) - SPONTANEOUS FLORA

Ioan Alexandru SĂRĂCIN[1], Ionel OBRETIN[1], Emilia CONSTANTINESCU[1], Mihnea GLODEANU[1], Patricia-Aida SĂRĂCIN[2], Ion SĂRĂCIN[1]

[1]University of Craiova, 13 A.I. Cuza Street, Craiova, Romania
[2]University of Medicine and Pharmacy of Craiova, 2 Petru Rares Street, Craiova, Romania

Corresponding author email: emiliaconst2000@yahoo.com

Abstract

Antioxidant activity is a vital parameter in assessing the potential health benefits and practical applications of natural products. This study investigates the antioxidant potential of Amaranthus retroflexus L. extracts using the 2,2-diphenyl-1picrylhydrazyl (DPPH) method. The DPPH method, widely employed for its simplicity and reliability, relies on the ability of antioxidants to neutralize the purple-colored DPPH radical, leading to a color change in the solution. By establishing a concentration-response relationship through testing various concentrations of Amaranthus retroflexus L. extracts, a dose-response curve was constructed, pivotal for determining the IC50 value, which represents the concentration capable of scavenging 50% of free radicals. Lower IC50 values indicate stronger antioxidant activity. The findings reveal variations in the inhibitory potential of Amaranthus retroflexus L. extracts, suggesting their potential utility in pharmaceuticals, agriculture, and other industries. This study underscores the significance of exploring natural sources for antioxidant compounds and provides valuable insights into harnessing the antioxidant properties of Amaranthus retroflexus L. for various applications.

Key words*: Amaranthus retroflexus L., half-maximal inhibitory concentration, concentration-response.*

INTRODUCTION

Amaranthus retroflexus L., popularly called the Red News, due to its valuable biological properties, rich phytochemical composition and extensive pharmacological activity, has become an area of widespread scientific and industrial interest.

In recent years, an increasing interest has been observed in plant raw materials, whose properties allow them to be used, both in food and for therapeutic purposes (Karamac et al., 2019). *Amaranthus retroflexus* L. is one of the most studied plants, as a natural source of a wide spectrum of biologically active compounds, relevant for reducing the risk of chronic diseases. This species has been well known since the time of the Aztecs, Mayans and Incas, spreading from the 16th-17th centuries to various other countries, as pseudo-cereals, vegetables, weeds or crops. *Amaranthus retroflexus* is a weed, with a cosmopolitan worldwide distribution (Baraniak and Kania-Dobrowolska, 2020). This species successfully invades and is adapted to a wide variety of climates (Alebrahim et al., 2021). The plant is native to North America and has become a naturalized weed in many countries, both in the northern and southern hemispheres (Weller et al., 2021). In Romania, it can be found throughout the country, in steppe areas, in the beech layer, especially on productive soils, fertilized with nitrogen. It is included in the list of weed species, causing losses to agricultural production (Dinu Mihaela et al., 2017).

This annual weed is occasional on cultivated land and in waste places such as garbage. It grows best at high temperatures and light intensities (Alebrahim et al., 2021), tolerating a wide pH range (4.2 to 9.1), although it is less common in acidic soils (Weller et al., 2021). The plant is widely used in the pharmaceutical industry, to produce drugs against atherosclerosis, gastric ulcers, tuberculosis, as well as as an antiseptic, antifungal and anti-inflammatory preparation. Amaranth seed oil exhibits hypolipemic, anti-atherosclerotic, hypotensive and antioxidant activity (Park et al., 2020). Therefore, its consumption can inhibit the development of food-related diseases

(Szwejkowska and Bielski, 2012). Since a significant content of squalene was found in Amaranth oil, this liver protective activity is due to this fact (Obiedzińska and Waszkiewicz-Robak, 2012). The oil can be used in the care of all skin types. It hydrates, soothes irritations, accelerates wound healing and has antimicrobial properties. It contributes to the regeneration, nourishment and strengthening of the epidermis, acting as an antioxidant (Lăcătusu et al., 2018). Some biochemical components - proteins, lipids, mineral substances, vitamins, are present in larger quantities in the spontaneous flora, compared to other cultivated species (Toader and Roman, 2007).

MATERIALS AND METHODS

The collection of samples was carried out from the seeds of the plants, harvested from the spontaneous flora, specific to the researched area (surroundings of Craiova). The material was identified in the Pharmacognosy laboratory of UMF Craiova, dried at ambient temperature. The collected samples are processed to extract the active compounds, which may be responsible for the observed inhibitory effects. Obtaining the alcoholic extracts: the dry product was ground in a grinder with walls made of stainless material, in order to obtain fine powders. 0.200 ± 0.001 g of each dry, finely ground plant material was weighed on an analytical balance, with three decimal places, and 5 mL of alcohol (methanol 70%, respectively ethanol 70%), brought in previously at temperatures of 70°C.

The extracts were either shaken (200 rpm orbital shaker Panasonic MIR-S100) or ultrasonicated (ASonic PRO50 bath), then centrifuged at 3500 rpm for 10 minutes, the supernatant was collected and brought to a total volume of 10 mL with MeOH 70% or EtOH 70%

The following samples were obtained:

• *Amaranthus retroflexus* seeds ethanolic extract - shaking (Amaranthus SEA)

• *Amaranthus retroflexus* seeds ethanolic extract - ultrasonication (Amaranthus SEU)

Ultrasound-assisted extraction is an extraction technique that uses high-frequency sound waves (ultrasound) to disrupt cell walls and enhance the release of phenolic compounds from plant materials (Michalaki et al., 2023).

Determination of antioxidant activity by the DPPH method

The method started from the preparation of a $0.25x10^{-3}$ Mol/L DPPH solution in methanol and a $0.25x10^{-3}$ M trolox solution in ethanol. From each alcoholic extract, 5 different concentrations were made: 10, 25, 50, 75, 100 and 200 µl/ml. 10 µl of extract from each extract concentration and 150 µl of DPPH were pipetted into 96-well microplates. The absorbance was determined at 515 nm (Tecan Infinite M1000 Pro), at 5, 10, 15 and 30 minutes after starting the experiment (Figure 1).

The percent inhibition of DPPH was calculated as follows:

PI (%) decolorization = [1 – (Abs Sample/ Abs Blank] 100 (1).

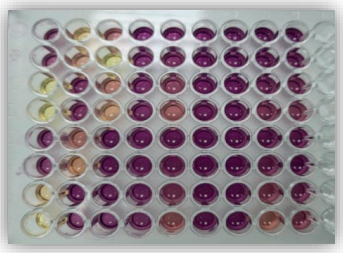


Figure 1. DPPH discoloration under the action of plant extracts of various concentrations

RESULTS AND DISCUSSIONS

Being in close correlation with antioxidant activity, the IC50 value (maximum inhibitory concentration 1/2) is the concentration of the sample capable of eliminating 50% of free radicals, in the DPPH (2,2-diphenyl-1picrylhydrazyl) method, for their evaluation. The study may reveal any variations in the inhibitory potential of *Amaranthus retroflexus* L., which may have implications for their potential use in different applications such as pharmaceuticals or agriculture. The concentration-response relationship is established by testing different concentrations of *Amaranthus retroflexus* L. extracts. These data are used to plot a dose-response curve, which is crucial for determining the IC50 value.

Antioxidant activity by the DPPH method

The DPPH method is a very widespread and simple method used to evaluate the antioxidant

activity of plant extracts or other types of samples (chemical compounds, food industry samples (La J, Kim MJ, Lee J, 2021). This method is based on the ability of antioxidant compounds to reduce the DPPH· radical, a purple-colored organic free radical (Figure 2), to an inactive, colorless form. The stronger the antioxidant activity of the sample, the faster reduction of the DPPH radical, implicitly a discoloration of the solution with plant extract (Iordănescu et al., 2021)

Antioxidant activity can be expressed as percent DPPH inhibition or as inhibitory concentration (IC50), which is the sample concentration required to halve DPPH· uptake. The lower the IC50 value, the stronger the antioxidant activity of the extract.

The strongest antioxidant activity was the methanolic extract obtained by ultrasound and shaking from Amaranthus seeds (Amaranthus SMU 37.81 µL extract/mL, Amaranthus SMA 57.16 µL extract/mL) (Table 1, Figure 3).

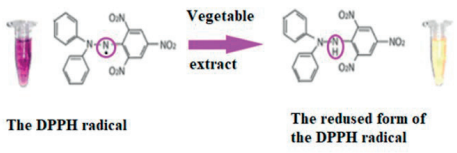


Figure 2. Reduction of the DPPH·radical to an inactive form

Table 1. Inhibitory capacity of extracts, expressed as IC 50 (µl extract/ml)

No.crt	Sample	IC 50 (µl extract/ml	Standard deviation n=3 determinations
1	*Amaranthus* SEA	86.43	0.42
2	Amaranthus SEU	76.23	0.25

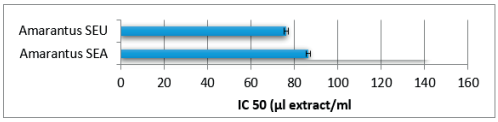


Figure 3. Inhibitory concentration of different types of extracts from *Amaranthus retroflexus* seeds

Table 2. 12/3-9 *Amaranthus retroflexus* seeds methanolic extract - stirring

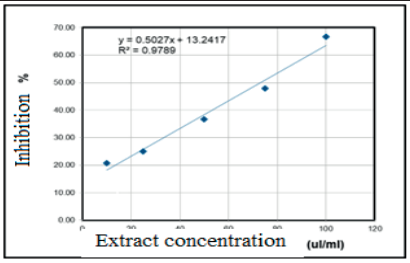
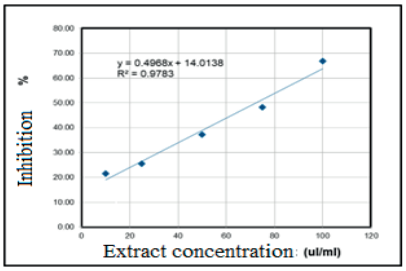
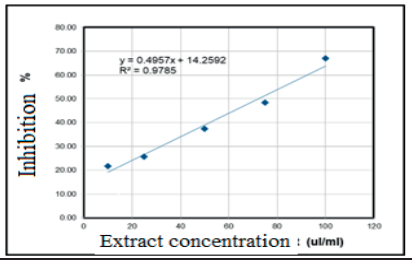
	IC 50 =(50-b)/a	a	b	r^2	
12_3-9 rehearsal 1	73.12+	0.5027	13.2417	0.9789	
12_3-9 rehearsal 2	72.44	0.4968	14.0138	0.9783	
12_3-9 rehearsal 3	72.10	0.4957	14.2592	0.9875	
	Average 72.55	SD 0.52			

Table 3. 12/3-10 *Amaranthus retroflexus* seeds methanolic extract - ultrasonication

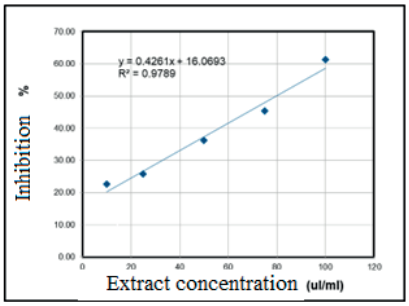
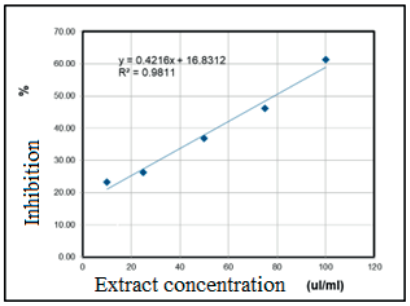
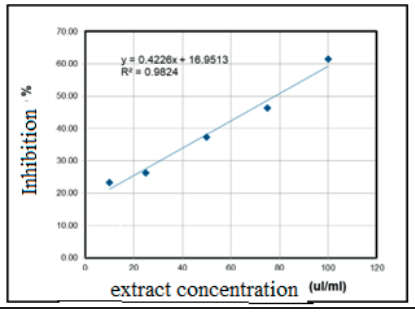
	IC 50 =(50-b)/a		a	b	r^2	
12_3-10 rehearsal 1	79.63		0.4261	16.0693	0.9789	
12_3-10 rehearsal 2	78.67		0.4216	16.8312	0.9811	
12_3-10 rehearsal 3	78.20		0.4226	16.9513	0.9824	
	Average	78.84	SD 0.73			

Table 4. 12/3-19 *Amaranthus retroflexus* seeds ethanolic extract - stirring

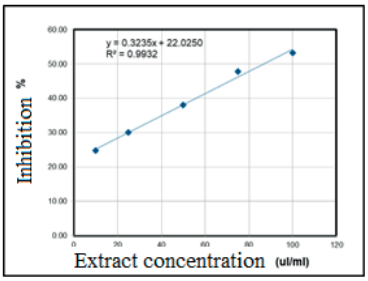
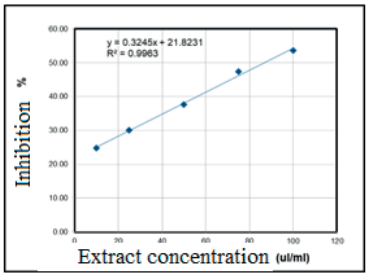
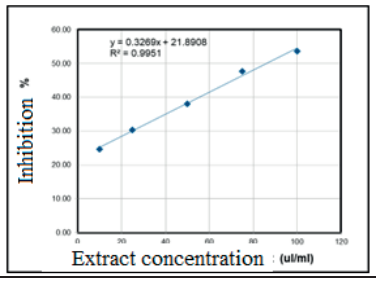
	IC 50 =(50-b)/a	a	b	r2	
12_3-19 rehearsal 1	86.48	0.3235	22.0250	0.9932	
12_3-19 rehearsal 2	86.83	0.3245	21.8231	0.9963	
12_3-19 rehearsal 3	85.99	0.3269	21.8908	0.9951	
	Average 86.43	SD 0.42			

Table 5. 12/3-20 *Amaranthus retroflexus* seeds ethanolic extract – ultrasonication

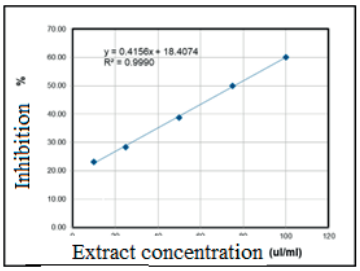
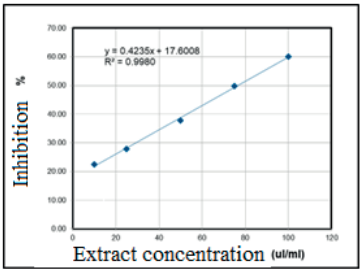
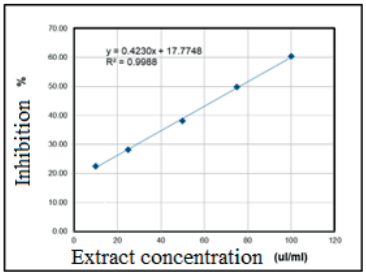
	IC 50 =(50-b)/a	a	b	r^2	
12_3-20 rehearsal 1	76.42	0.4156	18.4074	0.9990	
12_3-20 rehearsal 2	76.50	0.4235	17.6008	0.9980	
12_3-20 rehearsal 3	76.18	0.4230	17.7748	0.9988	
	Average 76.23	SD 0.25			

Table 6 shows a statistic of the inhibition capacity of the extracts at different time intervals. Figure 4 shows the inhibition of different types of extracts with the same concentration (100 µl/ml) at 5, 10, 15 and 30 minutes, respectively.

The seeds show increased inhibitory activity. In the vast majority of extracts, an increase in the inhibitory potency is observed through ultrasound, similar to the results obtained for total polyphenols.

Table 6. Determination of the inhibition capacity of the extracts, at various concentrations, over time

Concentration µl/ml	Average 5 min	SD	Average 10 min	SD	Average 15 min	SD	Average 30 min	SD
***Amaranthus retroflexus* seeds methanolic extract - stirring**								
10	**16.887**	**0.261**	**18.208**	**0.364**	**19.344**	**0.412**	**21.337**	**0.526**
25	**21.106**	**0.148**	**22.179**	**0.651**	**23.547**	**0.308**	**25.414**	**0.377**
50	**28.473**	**0.648**	**32.096**	**0.336**	**34.028**	**0.338**	**37.095**	**0.357**
75	**36.722**	**0.483**	**41.541**	**0.422**	**44.064**	**0.296**	**48.144**	**0.285**
100	**50.912**	**0.433**	**55.863**	**0.270**	**59.558**	**0.270**	**66.785**	**0.136**

***Amaranthus retroflexus* seeds methanolic extract - ultrasonication**								
10	**19.807**	**0.251**	**20.455**	**0.410**	**21.614**	**0.246**	**23.017**	**0.407**
25	**20.903**	**0.392**	**22.454**	**0.513**	**23.889**	**0.313**	**26.101**	**0.342**
50	**28.033**	**0.669**	**31.456**	**0.373**	**33.290**	**0.236**	**36.766**	**0.514**
75	**34.798**	**0.487**	**39.261**	**0.467**	**41.784**	**0.319**	**45.936**	**0.480**
100	**49.188**	**0.622**	**53.115**	**0.241**	**55.791**	**0.214**	**61.363**	**0.105**
***Amaranthus retroflexus* seeds ethanolic extract - stirring**								
10	**20.879**	**0.181**	**21.519**	**0.433**	**22.657**	**0.392**	**24.788**	**0.121**
25	**25.114**	**0.173**	**26.024**	**0.439**	**27.372**	**0.163**	**30.183**	**0.175**
50	**30.754**	**0.192**	**32.391**	**0.417**	**34.068**	**0.150**	**37.945**	**0.233**
75	**38.055**	**0.254**	**40.492**	**0.464**	**43.016**	**0.282**	**47.626**	**0.258**
100	**40.905**	**0.449**	**44.707**	**0.334**	**47.433**	**0.212**	**53.511**	**0.211**
***Amaranthus retroflexus* seeds ethanolic extract - ultrasonication**								
10	**19.600**	**0.124**	**19.946**	**0.334**	**20.971**	**0.118**	**22.730**	**0.321**
25	**23.346**	**0.052**	**24.405**	**0.372**	**25.357**	**0.193**	**28.134**	**0.260**
50	**31.095**	**0.094**	**32.563**	**0.305**	**34.675**	**0.180**	**38.169**	**0.460**
75	**37.860**	**1.003**	**41.939**	**0.363**	**44.682**	**0.141**	**49.855**	**0.123**
100	**45.545**	**0.297**	**50.256**	**0.392**	**53.329**	**0.327**	**60.129**	**0.157**

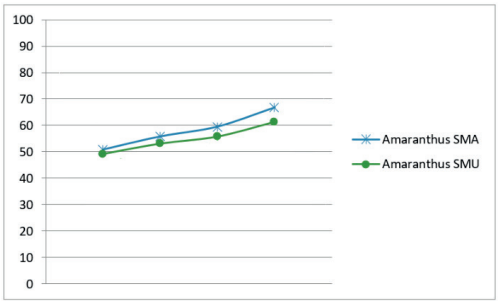


Figure 4. Time evolution of the inhibitory capacity at the concentration of 100 µl/ml

CONCLUSIONS

Comparing the two extractive methods (stirring and ultrasound) an increase in the extracted phenolic compounds using ultrasound is found. In this way, ultrasound can speed up the extraction process, reducing the extraction time compared to agitation. The yield of extractives using ultrasound is improved, while also ensuring the preservation of thermo-sensitive compounds.

However, we must take into account that there are also certain factors that can affect this type of extraction, namely the frequency or the type of sample, but also the fact that, the prolonged use of high-intensity ultrasound, can lead to the heating of the sample, which can affect the stability some phenolic compounds.

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