

Studying of the Antioxidant Capacity of Sweet Wormwood (*Artemisia annua* L.)

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In the last years, the research on the sweet wormwood (*Artemisia annua* L.) has increasingly become the focus of oncology science. The bioactive ingredient of the plant is artemisinin, which has been proven to be effective in the treatment of malaria. At the same time, Hungarian and international research groups are also investigating the plant, with the research aimed at the treatment of malignant cancer. In Europe, the therapeutic use of medicinal plants against tumours is realized in relatively few countries, in contrast, phytotherapy research in Asia reports results with a significant therapeutic effect. The aim of our work was to investigate the antioxidant effect of *Artemisia annua*. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Oxygen radical absorbance capacity (ORAC) laboratory assays proved that the parts of the herb show a significant antioxidant effect, while the seed and the extracted bioactive ingredient artemisinin have no antioxidant capacity at all. On the other hand, aqueous extracts made from leafy shoots showed promising antioxidant capacity values DPPH 10.48 ± 0.46 . Due to its bitter taste index 1548, it can be used in premixed feed e.g. for piglets.

Keywords: sweet wormwood (*Artemisia annua* L.), DPPH, ORAC, antioxidant capacity

1 Introduction

The sweet wormwood (*Artemisia annua* L.) is an annual, herbaceous, short-day herb, and belongs to Asteraceae family. It is mainly native to the temperate zone of Asia. Its genetic centre is in China. Because of its medicinal effects, it is now cultivated in many countries, which is why different species of the herb can also be found in America, as well as in Southern and Eastern Europe (Das, 2012; Bilal et al., 2014). *Artemisia annua* has been used in traditional medicine for many years in Asia and Africa for the treatment of malaria and fever, in the form of tea or pressed juice (Müller et al., 2000). *Artemisia annua* is also described to have anti-inflammatory and antitumour therapeutic effects (Kim et al., 2014; Ho et al., 2014; Efferth, 2017). Its stem is erect brownish or violet brown, the leaves of the herb have a length of 0.03–0.05 m and are divided by deep cuts into two or three small leaflets.

The intensive special aromatic scent of the essential oil of leaves is characteristic. The main compounds of the vegetative parts of the herb are monoterpenes, sesquiterpenes, cumarins, phenolic compounds and flavonoids (Gouveia & Castilho, 2013; Munyangi et al., 2020). In recent years, several papers have demonstrated the antioxidant activity of *Artemisia annua*, which could be due to the presence of phenolic compounds (Čavar et al., 2012; Messaili et al., 2020). Oxygen free radicals contribute greatly to the development of several types of cancer. At high concentrations, they generate oxidative stress. These are generally reactive oxygen species (ROS) that result from the cellular redox process (Halliwell & Gutteridge, 2015). Antioxidants are molecules that neutralize free radicals. They are bioactive substances that can be lipophilic (vitamin E, β -carotene, vitamin A), hydrophilic (vitamin C, amino acids, polyphenols),

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cytosolic antioxidants (coenzyme Q10), and structural antioxidants (trace elements, e.g. selenium, zinc, amino acids, selenocysteine), (Szent-Györgyi, 1928; Cornetti, 2009). The best antioxidants source are herbs or healthy fruits and vegetables (Dragsted et al., 2004). From this aspect the sweet wormwood can be a raw material of several dietary supplement or fodder premix products. Our aim was the studying of antioxidant activity of above-ground parts of *Artemisia annua*. After that the taste value of herb (bitter, sour and sweet) was investigated.

2 Material and methods

2.1 Method of Growing

Our plant testing research was carried out in 2021–2022. The sweet wormwood seedlings were raised in a heated growing greenhouse with the following parameters: night and day temperature 20–26 °C, air humidity 80%, regular irrigation. In both years, the planting took place at the end of May, plant to plant 1 × 1 m. The seedlings were characterized by a strong stem, on average 0.4 m high, and a richly branching leafy shoots. The plants were grown under open-field cultivation conditions; no additional nutrient supply, no chemical plant protection or other phytotechnical methods were used. We started cutting and collecting the plants at the beginning of October in both years. The fresh plants were stored under the temperature of 4 °C, in dark, before the laboratory investigation.

2.2 Method of Antioxidant Capacity Assay

The laboratory determination of the antioxidant capacity was measured at the Faculty of Pharmacy of the University of Szeged. DPPH was used to determine the values and a more modern ORAC method was used to proof this. The results of wormwood were compared with DPPH value of high antioxidant herb *Epilobium parviflorum* tea, man-made butyl hydroxytoluene (BHT) and natural quercetin extracted from tomato. All assays were in three repeats. The examined plant fractions were fresh leafy shoot vs. dry leafy shoot (tea), seed of *Artemisia annua* and 98% pure artemisinin bioactive ingredient.

2.3 Method of DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The essence of the method is that the purple DPPH free radical is reduced by the antioxidants in its environment, because of which its colour intensity decreases. At the end of the reaction, the antioxidant effect can be expressed by the EC_{50} value, which represents half of the amount of material required to exert complete inhibition, by examining the extent of the decrease in colour intensity with a spectrophotometer. Based on this, it can be said

that the lower the EC_{50} value, the higher the antioxidant capacity, because the less substance is needed to exert the effect (Frankel & Meyer, 2000).

2.4 Method of ORAC (Oxygen Radical Absorbance Capacity) Assay

The ORAC method was developed by Cao et al. (1993). During the measurement, the test molecule (protein) reacts with peroxy radicals (ROO) and suffers oxidative damage, therefore the intensity of the fluorescent signal it emits will decrease. This reaction is inhibited due to the effect of antioxidants. The peroxy radicals are obtained as a result of the thermolysis of the azo dye AAPH (2,2'-azo-bis(2-amidinopropane)-dihydrochloride). Reaction conditions: pH = 7.4; temperature 37 °C. During the assay, free radicals generated with AAPH are neutralized by the antioxidants in the environment, and the antioxidant capacity can be calculated from the fluorescence change during this process. The unit of antioxidant capacity is ($\mu\text{g.TE.l}^{-1}$) Trolox equivalent/litre value. The higher the value, the higher the antioxidant capacity, because a unit amount of sample corresponds to more Trolox (Cao, et al., 1993; Balogh, 2010).

2.5 Method of Determination of Bitter Substance of Drugs

In the case of drugs, the bitterness value refers to the greatest dilution of the aqueous extract of 1 g of the drug dried at 105 °C, a portion of which still produces the sensation of a bitter taste 10 ml in the mouth in half a minute, especially around the base of the tongue. The obtained result is subjective data that must be normalized, so we must define a correction factor (oral factor). The bitter taste sensitivity of the mouth is determined by different dilutions of the aqueous solution of quinine-hydrochloride. Thus, the determination of the bitter value is based on the comparison with quinine-hydrochloride (Rédei et al., 2015).

2.6 Student's t-test

For determination of differences between the obtained results of antioxidant activity the Student's *t*-test was applied. It is a one-sample location test of whether the mean of samples have a value specified in a null hypothesis. The general condition of *t*-tests is therefore that the samples are continuous variables from a normal distribution (Goulden, 1956; Beibei & Ying, 2017).

2.7 Method of Determination of TPC (Total Phenolic Content)

The total phenolic content was determined by the Folin-Ciocalteu method by Singleton and Rossi (1965). 0.125 ml

of extract was mixed with 1.8 ml of Folin-Ciocalteu reagent (10 fold diluted) and kept for 6 min at 25 °C. After this, 1.2 ml of 15% Na₂CO₃ was added to the reaction mixture and kept for 90 min at RT. The absorbance was measured at 765 nm. TPC assay of fresh herb was by HPLC used.

3 Results and Discussion

The antioxidant capacity of fresh and dry leafy shoots, seeds harvested in 2020 and 2021 were assayed by the DPPH method. After this, the ORAC method was used to prove the DPPH values (Table 1). The bioactive ingredient artemisinin was extracted from fresh herbs in both cultivation years. The Fig. 1 shows the DPPH value of fresh and dry wormwood leafy shoots compared with natural quercetin, artificial BHT and known high antioxidant dry leafy shoot of *Epilobium parviflorum* herb. The DPPH EC₅₀ (µg.l⁻¹) standard limit was from cherry shaped red pepper (*Capsicum annuum* L. cerasiforme) extracted vitamin C. This means that the samples have outstanding antioxidant efficiency, which are below the DPPH line of vitamin C. 100% DPPH antioxidant capacity means the neutralization of 1.14·10⁻⁴ mmol free radical (Hegedűs, 2013).

The results of the DPPH and ORAC assays clearly pointed out that the degree of antioxidant capacity is significantly different between the individual parts of the *Artemisia annua* herb. Based on the results of the individual measurements, it could be established that the pairwise analysis of the vegetative part (leafy shoot) and generative part (seed) of *Artemisia annua* according to the two measurement methods indicated a significant difference in their antioxidant effects even at the 0.1% probability level. Furthermore, a significant difference can be observed between the antioxidant

Table 1 Average values (2020–21) of antioxidant capacity of *Artemisia annua* herb methanol extract (n = 6)

Samples	DPPH EC ₅₀ (µg.l ⁻¹)	ORAC (mmolTE.g ⁻¹)
Fresh leafy shoot	10.48 ± 0.46	2.46 ± 0.13
Dry leafy shoot (tea)	13.65 ± 0.69	1.92 ± 0.03
Seed	45.42 ± 2.84	0.83 ± 0.01
Artemisinin	–	0.28 ± 0.18

capacities of the investigated plant parts, determined separately by both methods (DPPH and ORAC), even at the 0.1% probability level. At the same time, in the case of artemisinin ingredient, the DPPH method showed no antioxidant effect at all, while the ORAC method indicated an unimportant antioxidant effect (Table 1).

The fresh leafy shoots of *Artemisia annua* showed the most active antioxidant effect, but this is significantly lower than the antioxidant properties of *Epilobium parviflorum* herb (Fig. 1).

Several scientific publications stated that the antioxidant capacity

of hoary willowherb is due to its rich polyphenol composition (Steenkamp et al., 2006; Hevesi et al., 2009; Ferreira et al., 2010). From this aspect, we controlled the total polyphenol compound (TPC) of *Artemisia annua* leafy shoots.

The bitterness value is the reciprocal of the dilution of the still bitter-tasting compound, liquid or extract. This was determined by comparison with quinine hydrochloride, which has a bitterness value of 200,000. The average value of the determination of the bitterness value of the leafy shoots (fresh and dry) of the sweet wormwood (*Artemisia annua* L.) performed in 8-8 repetitions is 1.548.

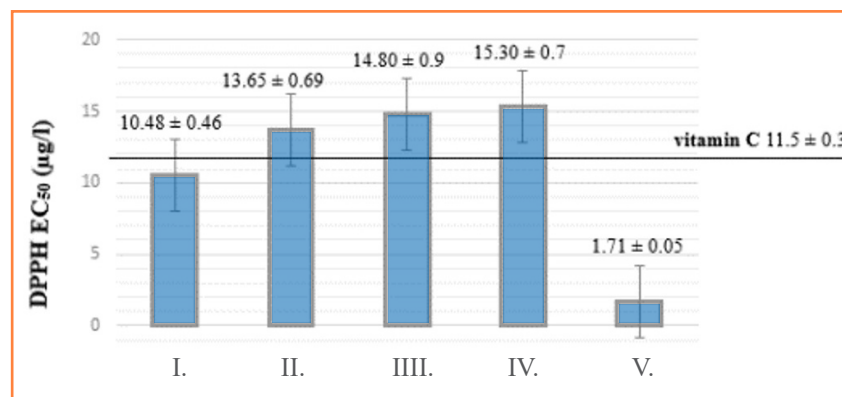


Figure 1 DPPH antioxidant capacity values (standard: vitamin C)
Samples: I. – fresh leafy shoot of *Artemisia annua*; II. – dry leafy shoot of *Artemisia annua*; III. – extracted quercetin; IV. – BHT; V. – fresh leafy shoot of *Epilobium parviflorum*

Table 2 TPC content of *Artemisia annua* and *Epilobium parviflora* methanol extract (n = 6)

Method Total Polyphenol Content (TPC)	Amount detected (mg.GAE.g ⁻¹ DW)
<i>Artemisia annua</i> fresh leafy shoot	23.30 ± 0.92
<i>Epilobium parviflora</i> fresh leafy shoot	134.50 ± 4.37

4 Conclusions

After summarizing our two years of growing experience, we can state that the sweet wormwood (*Artemisia annua* L.) herb can be grown under Hungarian meteorological and soil conditions. Based on the results of the antioxidant effect assay, we can prove that the leafy shoot of the herb has the greatest free radical scavenging effect. We attributed the neutralization of free radicals to the polyphenols content; this was confirmed by the comparison with *Epilobium parviflorum* herb. If we consider the vitamin C to be a proven strong antioxidant, then the above-ground vegetative parts of sweet wormwood have at least this character (Fig. 1).

The results of the determination of bitter substances, as well as other published studies, prove (Skowrya et al., 2014) that the herb *Artemisia annua* may be suitable for neutralizing free radicals, for example in the food matrix or in the production of animal (e.g. piglets) feeds (Brisibe & Umoren, 2009) to replace synthetic antioxidants. Based on our results, we recommend the herb for further pharmacognosy research.

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