A Comparative Study of the Antioxidant Activity of Garlic and Mistletoe Extracts

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Antioxidant are coumpounds with capacity to reduce toxic effect of free radicals , neutralizing instabil oxygen molecules. Previously made studies show presence of polyphenols in this plant , from this the idea to determine the antioxidant in these plants. After determination, done by TEAC, ORAC and DPPH method, from mistletoe and garlic extract, it becomes evident their capacity as antioxidant compound. Thus AVA has antioxidant action greater then E vitamin by TEAC method. By DPPH method , alcoholic mistletoe extract has better action then others.

Keywords: vitamina E, garlic, mistletoe, ascorbic acid

Natural antioxidants, found not only in fruits and vegetables,but also in medicinal plants neutralize free radicals, very important action in chronic diseases. Most important methods of determining antioxidant activity are: TEAC, ORAC,DPPH, FRAP [1].

Garlic, the oldest known herb on Earth, an important therapeutic agents, is also used for its antibiotic, antifungal and antibacterial propreties. The cardiovascular action of garlic can be explained by lowering the blood pressure thus having a cardioprotective effect on blood vassels which lowers the oxidative stress [2]. The antioxidative action of garlic is due to the sulfur compounds it contains, but also to allicin and other compounds. It is believed that the activity of garlic is dued to its sulfur derivates and compounds. The anticancer effect is dued in big part to allicine [3].

Viscum album, demi-parasite plant, has been used for centuries in traditional medicine for the treatment of cancer,epilepsy, infertility, menopause syndroms, nervous tension, asthma, hypertension. Mistletoe is rich in flavonoinds, amines, enzymes,minerals, polysaccharides, lectines. Experiments made by Elluru and collab. 2007 proove the anticancer effect of the miseltoe water extract on a cancer cell line [4].

The present work aims at highlighting the antioxidant activity of water and alcohoolic extracts of garlic and mistletoe using acknowledged methods in literature.

Experimental part

Materials and methods

Preparing the herbal material

Viscum album leaves that penetrate the bulbs and roots of *Alium sativa*. The branches of *Viscum album* were harvested in the Groseni area, county of Arad (Romania) on the month of June 2014, and the *Alium sativa* bulbs are from private culture harvested in the Groseni area. The herbal material, after being harvested has been dried fast for 48 h at the temperature of 90°C and kept in paper bags and boxes, in the absence of light and at the temperatureof 20°C [5, 6].

1. Processing the extracts

Two kinds of extracts were obtained:

a. Methanolic extracts:

0.2 g of dry material of (*Viscum album* and *Alium sativum*) were extracted with 70% methanol for 10 minutes at 70°C by stirring. After cooling down at room temperature, the extracts have been centrifugated at 200 rpm and filtered. Out of each filterred material 2mL were taken to which 100mL of H_2O has been added resulting *Visum album* estract (V1) and *Allium sativum* extract (V2) [7].

b. Water extract:

2 g of fresh material (*Visum album* and *Allium sativa*) have been stired with 10mL H_2O for 1 min after they were centrifugated at 10000 rpm for 10 min and the extract was filtered. The filtered materials are named: *Visum album* water extract (AVA) and *Alium sativum* water extract (AUA).

2. Determining the antioxidant capacity

Most used methods to determin the antioxidat capacity are TEAC method(the antioxidant capacity measured in Trolox equivalents), (ORAC) - *oxygen radical absorbance capacity* and (DPPH) (measuring the downfall of absorbtion of free radical DPPH maxim).

a.Determining the antioxidant capacity using the TEAC method-the antioxidant capacity espressed in Trolox equivalents

The method is based on the capacity of antioxidants to neutralize the ABTS radical anion.ABTS can be oxidated by peroxid radicals or other radicals to its cationic radical ABTS+, highly coloured, and its determination can be made using a spectophotometer at a wave length of 734nm.The antioxidant capacity is expressed as the potential of the tested compounds to decay the radical ABTS by reacting directly to it. (8,9).The antioxidant capacity of the tested compounds has been expressed as Trolox equivalents.

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The antioxidant capacity of the compunds was determined using the formula:

$$TEAC_{probă} = C_{Trolox} x f x + \frac{A_{probă} - A_{blank}}{A_{Trolex} - A_{blank}} \quad (1)$$

- A blank, maximum absorbance at 3 min after adding 2.5 mL ABTS in a tank containing 0.5 mL of distilled water;
- A Trolox, maximum absorbance at 3 min after adding 0.1 mL Trolox(10-3M), in a tank containing 2.5 mL Trolox and 0.4 mL distilled water;
- A sample, maximum absorbance at 3 min after adding 0.1 mL of sample (approximately 2mg/mL) in a tank containing 2.5mL ABTS and 0.4 mL of distilled water;
- f represents the dilution factor of the sample;
- C_{Trolox} representsthe effective concentration of Trolox measured in umol/-1.

b) The capacity of absorbtion of the (ORAC), radical of oxygen - *oxygen radical absorbance capacity.*

The ORAC method measures the oxidative degradation of a fluorescent sample(fluoriseine) after mixing it with a compound that is going to generate free radicals azo-compounds-like; Azo-initiators are compounds that produce radicals peroxil-like by heating them and thus deterriorate the fluorescent marker(the fluorescent molecule), so that a modification of the intensity of fluorescent emission occurs.The monitorisation is made cinetically every minute by using the spectophotometer. The stimulation is measured at 485 nm with a band of 30 nm, and the emission at 528 with a band of 20nm. The method was described by Huang at all 2002. The area under curve (AUC) and Net AUC of both standard and samples was determined using the ecuation:

$$AUC = \left(\frac{R_1}{R_1}\right) + \left(\frac{R_2}{R_1}\right) + \left(\frac{R_3}{R_1}\right) + \cdots + \left(\frac{R_n}{R_1}\right)$$

R1 - the value of the initial fluorescence
Rn - the value of fluoresence after 30min

NetAUC = AUCsample - AUCblank

The ORAC values were reported as Trolex equivalents, being expressed as umol TE/DW [10, 11].

c) DPPH method (2,2-diphenyl-1-picrylhydrazyl)

DPPH radical is widely used as a source of radicals to evaluate antioxidant activity,its monitorising being done using a spectophotometer at a wave length of 516 nm. During this process DPPH is going to act as free radical scavenger or hydrogen donor. The working method has been described by Molyneux in 2004, Milardovic in 2006 [12, 13].

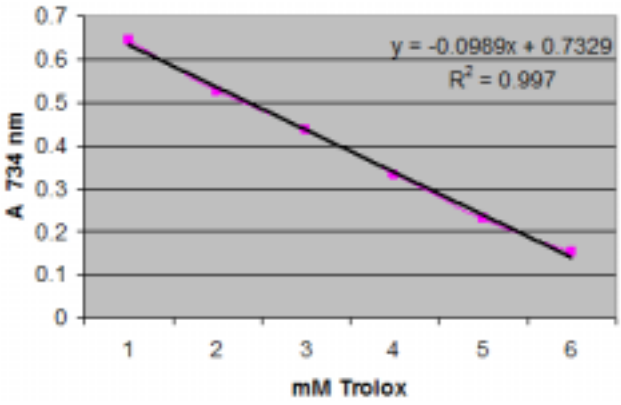

Fig 1.The etalonation curve for tocopherol (Vitamin E)

The antioxidant capacities of samples can be evidentiated from the absorbance curves of dependece as the report between the absorbance at time t(15) and the initial absorbance (t=0)

$$A\%(t) = \frac{A_{517nm}(t)}{A_{517nm}(t=0)} \cdot 100$$

The inhibition is calculated by using the formula:

$$I(\%) = \frac{A_{blanc} - A_{proba}}{A_{blanc}} \times 100$$

A_{blank}-the absorbtion of the sample;
A sample-the absorbtion of the vegetal compound

Results and discussions

a) Determining the antioxidant capacity using the TEAC method:

The antioxidant capacity of the mistletoe water extract (AVA) is superior to the antioxidant capacity of *Allium sativum*,the capacity of vitamin E being 83 mEquiv Trol/ vegetal material. As table 1 shows, the capacity of *Allium sativum* is 3 times higher than that of the standard antioxidant, vitamin E.

b)The (ORAC) capacity of absorbtion of the oxygen radical

The ORAC method for determining the antioxidant activity is probably the most used proton transfer method indicating the possibility of capturing free radicals over peroxyl radicals. The appropiate values (table 2) for the two extracts are the following:

Extracts	A 734nm	Concentration mMequivalent Trolox / g fresh vegetal sample
AUA	0.532	203.13±0.01
AVA	0.551	203.13±0.01

Table 1
THE ANTIOXIDANT ACTIVITY USING TEAC METHOD

Extracts	Antioxidant activity Mmol TE/DW
Viscum album extracts (AVA)	13.203 ± 0.4
Alium sativum extract (AVA)	12.516 ± 0.2

Table 2
ANTIOXIDANT ACTIVITY FOR MISTLETOE EXTRACTS (AVA) AND *ALLIUM SATIVA* USING ORAC METHOD

Extracts	Antioxidant capacity
U1	17.36
V1	27.51
AUA	14.77
AVA	14.60

Table 3
ANTIOXIDANT ACTIVITY OF PLANT EXTRA

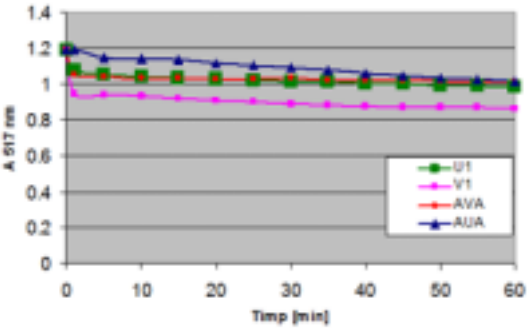

Fig. 2. DPPH oxidative capacity for vegetal extracts

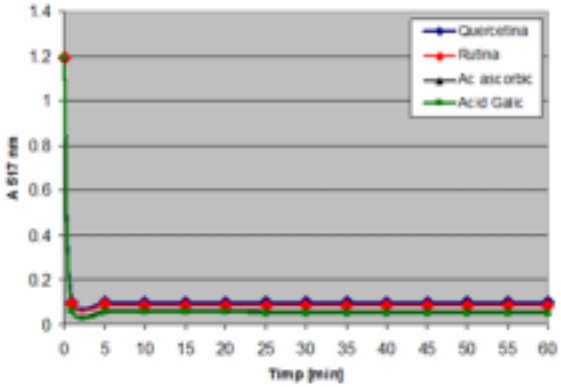

Fig. 3 The capacity of capturing DPPH for the standard

Antioxidant activity	Standard having the conc. 1mg/mL
91.02	Quercetin
92.86	Rutin
94.96	Ascorbic acid

Table 4
ANTIOXIDANT ACTIVITY OF STANDARDS

c) DPPH method(2,2, diphenyl-2-pycrylhidrazyl)

The antioxidative activity of alcoholic extracts (U1 and V1) and water extracts (AVA and AUA) is presented in table 3 and the variation of absorbance in time (the capacity to capture free radical) is depicted in figure 2.

The antioxidative activity for standards (quercetin, rutin, ascorbic acid and galic acid having the concentration of 1g/mL) is showed in table 4 and the variation of absorbance in time (the ability to capture free radical) is depicted in figure 3.

The antioxidant activity of standards varies accordingly:

Ascorbic acid > Rutin > Quercetin

The antioxidant action of extracts vary following the order:

AUA > AVA > U1 > V1

All the extracts have an antioxidant activity higher than 14%.

The antioxidant activity of the extracts is lower than that of the standards.

As a conclusion, the highest antioxidant activity is that of the mistletoe extract V1 and the lowest antioxidant activity is that of water extracts, both of mistletoe and garlic (AVA and AUA)

Conclusions

The results of this study show that the *Viscum album* extract has the highest antioxidant activity. This antioxidant activity is the result of the presence of hidroxyl groups in the flavonoid structure. All the extracts have an antioxidant activity that difers according to the type of extract(water or alcoolic), and to the material used in the extraction process. The alcoolic extract of Viscum album has a higher antioxidant activity than that of a-tocopherol. Garlic water extract has a net superior activity to that of vitamin E. The antioxidant activity of these extracts makes them a potential source of natural oxidants that could be incorporated in different pharmaceutical forms.

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