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RESEARCH ARTICLE

ROMANIAN AROMATIC PLANTS AS SOURCES OF ANTIOXIDANTS

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Abstract

Nowadays the concept of chemical preservatives in foods is very controversial. Consumers' choices redirect towards healthier and more natural alternatives. Spices and herbs have been used not only for flavoring food but also for improving the overall quality of the product and to extend the shelf-life of foods. The possibility of using some Romanian aromatic plants, oregano (*Origanum vulgare*), tarragon (*Artemisia dracunculus*), wild thyme (*Thymus serpyllum*), as a new source of natural antioxidant has been investigated using extraction with ethanol.

Many in vitro methods have been developed to evaluate antioxidant activity. The most widely used ones being the DPPH, ABTS, FRAP, ORAC assays. These assays are time-consuming, and when pro-oxidant compounds are to be evaluated, it suffers serious invalidity, especially in the presence of catalycally active iron. Total polyphenol content of the plant extracts was measured based on the Folin-Ciocalteu method. The antioxidant activity of the extracts was determined using PHOTOCHEM®, from Analytik Jena (Germany). The analytical method used in this study, the PCL assay, was chosen because it is rapid, sensitive, relatively simple and reproducible.

The result may suggest that the plant extracts possess compounds with antioxidant activity, and therefore can be used as natural preservative ingredients in food industry.

Keywords: antioxidant capacity; phenolic antioxidants; photochemiluminescence

Introduction

Although oxygen is the most important element for aerobic life, it has also been shown, however, to participate in a number of toxic chemical reactions. In particular, lipid peroxidation is a toxic reaction that commonly occurs in food via organoleptic deterioration during processing, distribution, and later storage stages (Vági *et al.*, 2005). Antioxidants are important in the food industry not only because of their usefulness as a preservation method but also because of their beneficial effects on human health. The use of synthetic antioxidants is limited because consumers are increasingly demanding additive-free or natural products (Babovic *et al.*, 2009). Phenolic antioxidants have been shown to play important roles in delaying the development of chronic diseases such as cardiovascular diseases (CVD), cancer, inflammatory bowel syndrome and Alzheimer's disease. Phenolic antioxidants are products of secondary metabolism in plants and are good

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sources of natural antioxidants in human diets (Chun, Vattem, Lin & Shetty, 2005).

Over the past 10 years, researchers and food manufacturers have become increasingly interested in polyphenols. Two aims of research are to establish evidence for the effects of polyphenol consumption on health and to identify which of the hundreds of existing polyphenols are likely to provide the greatest protection in the context of preventive nutrition. If these objectives are to be attained, it is essential to determine the nature and distribution of these compounds in our diet (Manach, Scalbert, Morand, Rémésy & Jiménez, 2004)

It is important to determine the amounts and species of polyphenols in plants, fruits and teas. The number of polyphenols has been estimated to be over one million, because they generally occur as glycosides, and the sugar species and binding forms show great variety. However, the bioactivity is attributed to aglycon structures, not to sugar moieties. The antioxidant potential is due mainly to the orthodiol (catechol) structure in aglycons (Sakakibara, Honda, Nakagawa, Ashida & Kanazawa, 2003).

Many in vitro methods have been developed to evaluate antioxidant activity. Unfortunately, these in vitro methods often do not correlate with the ability of compounds to inhibit oxidative deterioration of foods. This is because the activity of antioxidants in food systems depends not only on the chemical reactivity of the antioxidant (e.g., free radical scavenging and chelating) but also on factors such as physical location, interaction with other food components, and environmental conditions (e.g., pH). One of the major factors affecting the activity of antioxidants that scavenge free radicals in foods is their partitioning behavior in lipids and water. The tendency for lipophilic antioxidants to work best in foods with high water content, whereas polar antioxidants are most effective in bulk oil, has been termed the "antioxidant paradox" (Decker, Warner, Richards & Shahidi, 2005).

The number of methods for measuring the antioxidant capacity of foods, nutraceuticals, and other dietary supplements has increased considerably. Because of the complexity of the composition of foods and of the plant extracts, the investigation of each single antioxidant compound is costly and inefficient; moreover, possible synergistic interactions among the antioxidant compounds in the food mixture or plant extract are not taken into account (Zielinska, Szawara-Nowak, Ornatowska & Wiczkowski, 2007). The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity. The presence of antioxidants leads to the disappearance of these radical chromogens; the most widely used ones are the ABTS and DPPH methods. Some other commonly used assays like FRAP assay, ORAC assay, PCL assay are mentioned below (Ali *et al.*, 2008). The DPPH free radical (DPPH) does not require any special preparation, while the ABTS radical cation ($ABTS^{\cdot+}$) must be generated by enzymes or chemical reactions. Another important difference is that $ABTS^{\cdot+}$ can dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidant (Wojdylo, Oszmianski & Czemerys, 2007). ORAC (Oxigen Radical Absorbance Capacity Assay) measures antioxidant inhibition of peroxyl-radical-induced oxidations and reflects classical radical chain-breaking antioxidant activity by H-atom transfer. FRAP assay is based on the ability of phenolics to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) by the action of electron-donating antioxidants (Karadag, Ozcelik & Saner, 2009). However, this assay is time-consuming, and when pro-oxidant compounds are to be evaluated, it suffers serious invalidity, especially in the presence of catalytically active iron (Cheng, Yan, Li & Chang, 2003)

In the PCL (photochemiluminescence) assay, the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. The PCL is based on the

inhibition of the photo induced autoxidation of luminol by antioxidants, mediated by the radical anion superoxide ($O_2^{\cdot-}$) and is suitable for measuring the radical scavenging properties of single antioxidants as well as more complex systems in the nanomolar range (Zielinska *et al.*, 2007). Luminol works as photosensitier as well as oxygen radical detection reagent. The antioxidant potential is measured by means of the lag phase at different concentrations, calculated by a Trolox calibration curve expressed as mmol equivalents in antioxidant activity of a reference compound (i.e. Trolox)(Besco et al., 2007). The PCL assay, which is easy and rapid to perform, and although less reliable with lipophilic substrates, presents numerous advantages: it does not requires high temperatures to generate radicals and it is more sensitive to measurement, in few minutes, the scavenging activity of antioxidants against the superoxide radical which is one of the most dangerous reactive oxygen species (ROS) also occurring in human body (Besco et al., 2007).

The aim of the study was to determine the antioxidant capacity of selected plants of Romania origin commonly consumed by the Romanian population as an important constituent of their traditional food. Little is kwon about antioxidant potential of Romanian aromatic plants. For this purpose three plant extracts obtained from three aromatic plants, oregano (*Origanum vulgare*), tarragon (*Artemisia dracunculus*) and wild thyme (*Thymus serpyllum*), were studied in order to assess their total phenolic content and antioxidant activity. The total phenolic content was determined by the Folin-Ciocalteu method. The analytical method used in this study, the PCL assay, was chosen because it is rapid, sensitive, relatively simple and reproducible, making it an attractive biomonitoring tool especially for plant extracts, food supplements, nutrition and food technologies.

Materials and methods

Materials

Plant samples. Three plant materials dried, belonging to three botanical families, which are commonly consumed in Romania, were chosen for this study: oregano (*Origanum vulgare*), tarragon (*Artemisia dracunculus*) and wild thyme (*Thymus serpyllum*). The plant samples were obtained from a herbalist's shop in Galati, Romania (dried using a traditional method, as follows: once collected, the plant was ventilated to remove humidity, covered with a blanket to avoid sunlight and let dry in a ventilated place for 20-30 days, at 20 ˚C, depending on the season) (Ramirez, Senorans, Ibanez & Reglero, 2004)

*Solvents and chemicals***.** Gallic acid and ethanol were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Standard Folin-Ciocalteu's phenol reagent and anhydrous sodium carbonate were obtained from Merck (Darmstadt, Germany). ACL (Antioxidant Capacity of Liposoluble substance) kits, Trolox ((S)-(2)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were purchased from Analytik Jena AG, Jena, Germany. Ultra pure water was used for the experiments.

Sample extraction

Dry plants were extracted by varying concentrations (0-95%) of ethanol. Among various extracts the highest phenolic containing extracts (70% ethanol) were chosen for further analysis.

10 g of plant were brought to 100 mL 70% ethanol. The mixture was left 24 hours for maceration at room temperature (20 ˚C) and then put in an ultrasonic bath at 60 °C, 2h. The suspension was then vacuum filtered through a ceramic filter with the porosity of 40 µm and centrifuged at 10.000 rpm, 15 min. The ethanol was completely removed by vacuum concentrator (Martin Christ), at 50 °C to give a viscous mass. The crude extracts were stored at 0-4 °C before analysis.

Determination of Total Polyphenol Content

The total polyphenol content (TPC) was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1 (Anesini, Ferraro & Filip, 2008). Briefly, 1.0 mL of the diluted sample extract was transferred in duplicate to separate tubes containing 5.0 mL of 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room

temperature for 60 min before absorbance was measured against water at 765nm. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g material. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 µg/mL.

Determination of the Antioxidant Capacity of Lipid-Soluble (ACL) Compounds by the Photochemiluminescence (PCL) Method

PCL analysis for determining the antioxidant capacity of the plant extracts was carried out as described by (Popov & Lewin, 1996) and (Analytik Jena, 2004) with a Photochem apparatus (Analytik Jena, Leipzig, Germany) against superoxide anion radicals generated from luminol, a photosensitizer, when exposed to UV light. The antioxidant capacity of 70% ethanol extracts of plants was measured using ACL kits provided by the manufacturer designed to measure the antioxidant activity of lipophilic compounds. In ACL studies, the kinetic light emission curve, which inhibits no lag phase, was monitored for 180 s and expressed as micromoles of Trolox per gram of dry matter. The areas under the curves were calculated using PCL soft control and analysis software. As greater concentrations of Trolox working solutions were added to the assay medium, a marked reduction in the magnitude of the PCL signal and hence the area calculated from the integral was observed. This inhibition was used as a parameter for quantification and related to the decrease in the integral of PCL intensities caused by varying concentrations of Trolox. The observed inhibition of the signal was plotted against the concentration of Trolox added to the assay medium. The concentration of the added extract solution was such that the generated luminescence during the 180 s sampling interval fell within the limits of the standard curve (Zielinska *et al.*, 2007).

Preparation of the Trolox standard solution (ACL protocol). A volume of five hundred microlitres of Reagent 1 (kit ACL, Analytik Jena) were added to the vial containing Trolox (Reagent 4, kit ACL, Analytik Jena) and mixed by vortex for 20-30 s. The obtained stock solution contains 1 nmol Trolox as calibration standard. Measurements were done using volumes of 5, 10, 20 and 30 µL of the sample, and were repeated two times.

Results and discussion

The purpose of this study was to quantify and compare the antioxidant capacities of three plants from Romania. Indeed, an increasing interest in the health benefits of plants has led to the inclusion of plant extracts in dietary supplements and functional food.

In Table 1 the values of total phenolic contents are presented.

Table 1. Total phenolic content of ethanolic extracts

Plant	**Total phenolic content (g of GAE/100 g of DW [a])[b]**
Oregano *(Origanum vulgare)*	6.70 ± 0.64
Tarragon *(Artemisia dracunculus)*	5.50 ± 0.31
Wild thyme *(Thymus serpyllum)*	3.11 ± 0.08

[a] Dry weight

[b] All values were the mean of two measurements and expressed as mean ± SD

Oregano (*Origanum vulgare*), tarragon (*Artemisia dracunculus*) and wild thyme (*Thymus serpyllum*) accumulate large amounts of phenolic compounds. In accordance with the dry weight of the extract, the total phenolic content of studied plant extracts decreased as follows: oregano (*Origanum vulgare*) > tarragon *(Artemisia dranunculus)*> wild thyme *(Thymus serpyllum).*

Photochem® apparatus and method allow a precise determination of the antioxidant capacity of lipid-soluble (ACL) compounds and are efficient in time and cost (Apati *et al.*, 2003). Free radicals are generated in the measuring system itself by means

of photosensitizer. The free radicals were detected by their reaction with the chemiluminogenic substance. Luminol played double role as photosensitizer and also as oxygen radical detection reagent. In the presence of substances in plant extracts acting as radical traps, the intensity of the PCL was attenuated according to the concentration. In this way, the antiradical properties of the analyzed substances could reliably be quantified.

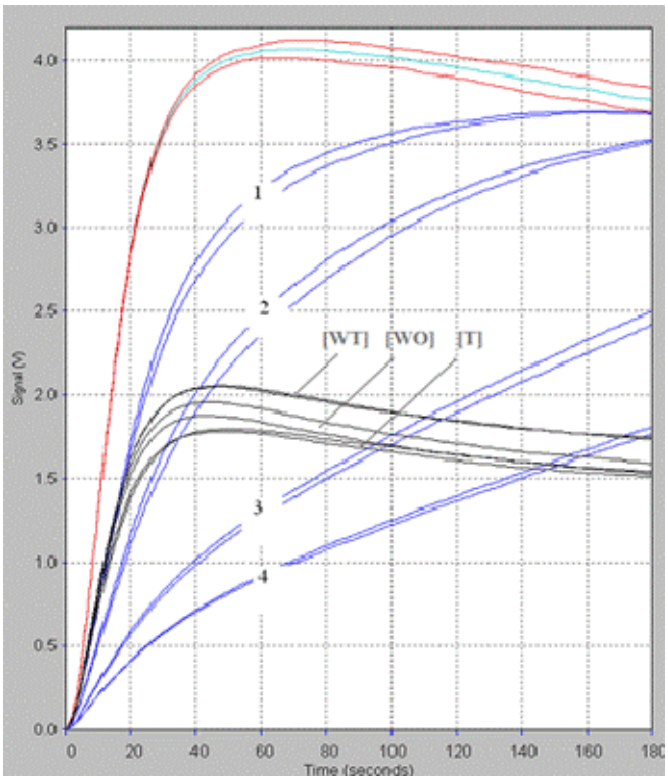

Figure 1. Antioxidant activity of lipid-soluble components of three plant extracts as measured by photochemiluminescence ([WT] –whild thyme, [WO] - oregano and [T]-tarragon. 1-4 trolox standards: 5, 10, 20, 30 µL)

Antioxidant activities measured using PCL methods are shown in Table 2 and Figure 1. The results for aromatic plants are presented in equivalent concentration units of trolox (Table 2).

Table 2. Lipid soluble antioxidant capacity, expressed as µg equivalents of Trolox for each gram of tested plant

Plant	**Trolox equivalents (µg/g plant)[a]**
Oregano *(Origanum vulgare)*	1.36 ± 1.12
Tarragon *(Artemisia dranunculus)*	0.91 ± 0.45
Wild thyme *(Thymus serpyllum)*	1.27 ± 0.07

[a] The values are mean of 2 measures ± SD

Differences between the results were likely due to genotopic and environmental differences (namely, climate, location, temperature, fertility, diseases and pest exposure) within species, choice of parts tested, time of taking samples.

The strong antioxidant activity of oregano (*Origanum vulgare*) provided from Romania was expected on the basis of the literature (Exarchou, Nenadis, Tsimidou, Gerothanassis, Troganis & Boskou, 2002), (Wojdylo *et al*., 2007), (Shan, Cai,

Sun & Corke, 2005), (Ivanova, Gerova, Chervenkov & Yankova, 2005).

The obtained results showed that oregano (Origanum vulgare), tarragon (Artemisia dracunculus) and wild thyme (Thymus serpyllum) were rich in phenolic constituients and had a good antioxidant capacity. The results proved the importance of phenolic compounds in the antioxidant behavior of spice extracts and also that they contribute significantly to the antioxidant activity of lipid-soluble components. Therefore, qualitative and quantitative analysis of major individual phenolics in the spices could be helpful for explaining the relationships between total antioxidant capacity and total phenolic contents in the species

Conclusion

In this study, the total phenolic content and antioxidant capacity of three different plants from Romania were measured. The results showed the high phenolic content and antioxidant capacity and provide useful information like the potential use of plants as a natural source of antioxidants and as a value-added product in the preparation of functional food ingredients and/or for enrichment of certain products.

Providing a reliable analytical method to detect the antioxidant capacities at very low concentrations of antioxidant in the sample is of major interest. Photochemiluminescence analysis provides many advantages over the other methodologies. It is simple, quick, sensitive, economical, convenient, and reliable.

These results support the possibility that these plants, which are commonly used in the Romanian diet as condiments or decoctions, can show protective effects on human health. However, further investigations in *in vivo* experiments and the absorption and metabolism of plants' bioactives are still necessary to further shed light on their efficacy in disease risk reduction.

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