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ANTIOXIDANT POTENTIAL OF BULGARIAN YARROW AND THYME

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Abstract. Phenols (sometimes called phenolics) are synthesized by plants for their general defense and particularly in response to antioxidative stress. These biologically active compounds are well known to have beneficial effects on human health. The aim of the present study was to apply a fast in vitro approach to compare the antioxidant potential of yarrow (*Achillea millefolium* L.) and thyme (*Thymus vulgaris* L.) that were freshly collected from their natural habitat in the Rodopi Mts with some randomly selected herbs for infusion from traders in Sofia region. Ethanol extracts from yarrow and thyme plants were compared with an extract from the Chinese white tea (*Camellia sinensis* (L.) Kuntze) used for infusion and known for its high phenolic content with antioxidant effect. The total quantity of phenols in both studied herbs varied in a close range but was 5-8 times lower than this in the white tea. The average antioxidant activity in thyme was slightly higher than in yarrow but was nearly four times lower in comparison to their activity in the white tea. Slight variations between the herbs from the Rodopi Mts and Sofia were found in the phenolic content of yarrow and thyme, and in the antioxidant activity of thyme. Significant positive correlation between the content of phenolic and antioxidant activity was observed in white tea and thyme, but not in yarrow but our in vitro approach would need to be confirmed by further in vivo analyses. Our results show that the genotype, habitat and storage conditions could influence the plant antioxidant potential and that it is likely that the Bulgarian herbs contain additional classes of metabolites which determine distinct biological activities.

Key words: antioxidant activity, herbs, phenolics, tea, white tea

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INTRODUCTION

The secondary plant metabolites serve as reducing agents that result in decrease of the amount of active oxygen species inside the cell, which prevents further damages and cell malfunction (Gupta & Sharma 2006; Pereira et al. 2013). The phenols (sometimes called phenolics) are secondary metabolites, widespread in herbal plants, and often studied for their antioxidant potential. The variable biological properties of the phenolics are due to their molecular structure including at least one phenol ring in which the hydrogen is usually replaced by a more active residue, such as hydroxyl, methyl or acetyl. These compounds often contain more phenolic rings, therefore they are called polyphenols (Dzialo et al. 2016). Plants synthesize phenolic compounds mainly to protect themselves against unfavourable environmental conditions such as ultraviolet light, herbivores and pathogens, as well as to attract pollinators and animals dispersing the seeds (Boudet 2007). The defence costs are paid mainly in the form of energy, carbon, and nitrogen, while phenolics are suggested to be cheaper than alkaloids because of the additional effort that is required to make inorganic nitrogen bioavailable (Mithöfer & Boland 2012).

Polyphenols are the subject of increasing scientific interest as they have various functions in the human body - antioxidant protection, anti-viral, antibacterial, anti-tumor and anti-inflammatory activity (Pandey et al. 2009). Epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases (Pandey et al. 2009). The teas made from *Camellia sinensis* (L.) Kuntze contain polyphenols and flavonoids (mainly catechins), which are considered as their most important phytochemicals in terms of health benefits due to their ability to act as antioxidants by donating electrons or hydrogen protons to reactive oxygen or nitrogen species (Shannon et al. 2018). White teas have been reported to possess higher antielastase, anticollagenase, and antioxidative activity than green tea, which has led to an increased interest in this tea type (Thring et al. 2009). In comparison, tisanes derived from herbs or fruit infusions, as chamomile and berry/hibiscus, also contain polyphenols but at significantly lower levels than *C. sinensis* derived teas.

Ethnobotanical studies highlighted the members of the Asteraceae and Lamiaceae families among the most popular medicinal plants in Bulgaria and other countries on the Balkan Peninsula, with different species of yarrow (*Achillea* L.) and thyme (*Thymus* L.) as commonly used herbs (*e.g.* Evstatieva et al. 2007; Jarić et al. 2015). The application of these medicinal plants in food industry, cosmetology and pharmacology has been increasingly studied (Mekinić et al. 2014; Boutaoui et al. 2018). Previous research work on these plants has been mainly confined to their essential oil, however, much attention has recently been directed to the water-soluble components (Benetis et al. 2008; Kratchanova et al. 2010; Mekinić et al. 2014; Rogova et al. 2015; Boutaoui et al. 2018). In yarrow, phenolic compounds such as flavonoids (*e.g.* vicenin-2, luteolin-3',7-di-O-glucoside, luteolin-7-O-glucoside, rutin, apigenin-7-O-glucoside, luteolin, apigenin) and phenolic acids (*e.g.* rosmarinic acid, m-hydroxybenzoic acid, o-coumaric acid, caffeic, ferulic acid) constitute one of the most important groups of pharmacologically active substances (Benetis et al. 2008; Mekinić et al. 2014). Recent investigation on bioactive substances in thyme species also revealed high content of phenolic compounds (benzoic acid, epicatechin, chlorogenic acid, syringic acid, naringin, catechin, o-coumaric acid) - Boutaoui et al. (2018).

Plant extracts made with water are nutritionally more relevant since herbs are traditionally ingested as hot-water infusions. However, stronger polar solvents (methanol, acetone, ethanol) are preferred for more exhaustive extraction of polyphenol compounds due to their polar groups (Benetis et al. 2008; Kratchanova et al. 2010). Kratchanova et al. (2010) investigated the influence of the extraction agent on the extractability of polyphenol components and the antioxidant activity of 25 Bulgarian medicinal plants, among which *A. millefolium* and *T. vulgaris*. It was found that the antioxidant potential was higher for 80% acetone extraction than for water extraction.

The phytochemical composition of medicinal plants is influenced by variables such as cultivar, ontogenetic factors, growth conditions, processing conditions, storage (Fiehn 2002; Kapchina et al. 2014; Boutaoui et al. 2018; Shannon et al. 2018). In the present study, we applied a simple preliminary, but fast *in vitro* approach to examine the total quantity of phenolic compounds and antioxidant activities of yarrow and thyme herbs from two different regions in Bulgaria, and compared them to the antioxidant potential of Chinese white tea, when using ethanol as a solvent.

MATERIALS AND METHODS

Plant material

The plant material was collected on 24th June 2017, from a natural habitat in Bulgaria, the Rodopi Mts (Plovdiv Province, village Dryanovo, latitude 41.7946091; longitude: 24.7867012; altitude 1000 m a.s.l.). The voucher specimens were deposited in the Herbarium of Sofia University „St. Kliment Ohridski", as follows: SO107842 for *Achillea millefolium* L. (yarrow) and SO107844 for *Thymus vulgaris* L. (thyme). Flowers were air dried at room temperature in darkness until no significant change of the dry weight was detected. The samples were analyzed four weeks after the collection. The commercially purchased yarrow and thyme herbs were randomly selected from Bulgarian producers in Sofia region (at average altitude of 500 m a.s.l.). The white tea (*Camellia sinensis*) consisted of unopened buds and it was purchased from a herbal pharmacy in Sofia in 2017.

Preparation of extracts

For extract preparation 50 mg of air-dried plant material was homogenized with 5 ml 100% ethanol and disintegrated in ultrasonic bath for 2 min. After centrifugation (at 9000 rpm for 20 min) the supernatant was subjected to further analyses.

Total phenolic content analysis

The total phenolic content was determined according to Singleton et al. (1999). Test samples contained 0.1 ml plant extract, 1.5 ml Folin-Ciocalteau reagent (previously dissolved in distilled water 1:10), 1.4 ml 7.5% Na_2CO_3. The samples were incubated in darkness, at room temperature for 30 min. The absorbance was measured at λ = 765 nm by spectrophotometer Shimadzu UV 1800. Standard curve based on known concentrations of gallic acid (GA) was used to calculate the amount of phenolic compounds as GA equivalents per dry weight (mg GA.g^{-1} DW).

Total antioxidant activity analysis

The total antioxidant activity of each extract was measured according to Prieto et al. (1999). Each sample contained 0.25 ml extract and 2.5 ml reagent solution (0.6 M H_2SO_4, 28 mM CH_3COOK and 4 mM $(NH_4)_6Mo_7O_{24}$). The samples were incubated in a water bath for 90 min at 95°C. The reaction was stopped by placing the samples on ice. The absorbance was measured at λ = 695 nm by spectrophotometer Shimadzu UV 1800. The total antioxidant activity is calculated according to Prieto et al. (1999) by multiplication with a coefficient from a standard curve with known concentrations of α-tocopherol and expressed as mM α-tocopherol per DW (mM.g^{-1} DW).

Statistical analysis

The shown values are mean values of six to nine measurements (three extractions with two-three technical repetitions) and the related standard deviation. The *t*-test was applied for statistical evaluation with a threshold $P < 0.05$. For the correlation analysis, first, linear regression analysis was applied after checking the assumptions for normality and equality of the variances. Next, Pearson Product Moment Correlation coefficient (r) was calculated with $P = 0.05$ accepted as a level of significance. Data analysis was made by SigmaPlot software.

RESULTS

The maximal phenolics content of yarrow and thyme ranged in close limits (28.0 -47.0 mgGA.gDW^{-1}). However, there were statistically significant differences between the material from the Rodopi Mts and Sofia regions. In yarrow, the phenolic content was higher in the plants from the Rodopi Mts in comparison to the plants from Sofia region (35.8 and 28.0 mgGA.gDW^{-1}, respectively). By contrast, in

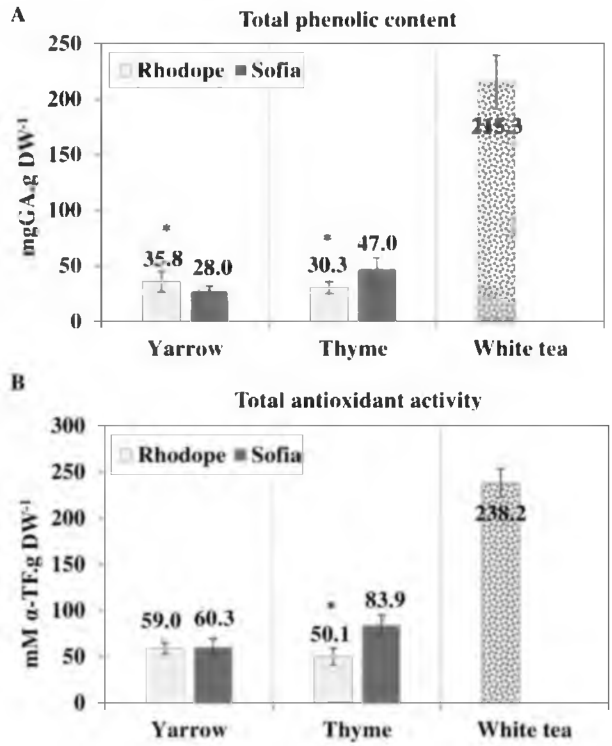

Fig. 1. Antioxidant potential of medicinal plants. Total content of phenolic compounds (A) and total antioxidant activity (B). Each variant from Rodopi Mts is compared to the one from Sofia region, and the presence of statistical difference is indicated with asterisks (* $P<0.05$; n>6).

thyme, the phenolic content was higher in the plants obtained from Sofia region (30.3 and 47.0 mgGA. gDW^{-1}, respectively; **Fig. 1A**).

The maximal antioxidant activity in thyme was slightly higher than this in yarrow (83.9 and 60.3 mM α-TF.gDW^{-1}, respectively; **Fig. 1 B**). There was no difference between the antioxidant activity of the yarrow samples from the Rodopi Mts and Sofia, while in thyme a higher antioxidant potential was found in the material from Sofia **(Fig. 1A, B)**.

The total phenolics content in the Chinese white tea was five to eight times higher in comparison to the studied yarrow and thyme material. Similarly, its antioxidant activity was four times higher **(Fig. 1, 2)**.

Statistically significant Pearson correlation was established between the amount of phenolics and the antioxidant activity in thyme plants, from both studied regions (r=0.908; $P < 0.001$) and in the white tea (r=0.999; $P < 0.03$), as well. By contrast, in the case of yarrow such correlation was not established.

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0%	50%	100%

		Total content of phenols relative to white tea	Total antioxidant activity relative to white tea
Yarrow	Rhodope	17%	25%
	Sofia	13%	25%
Thyme	Rhodope	14%	21%
	Sofia	22%	35%
White tea		100%	100%

Fig. 2. Heat map data representation of the antioxidant potential of Bulgarian yarrow and thyme relative to the white tea.

47.0 mgGA.gDW^{-1}, respectively; **Fig. 1A**).

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DISCUSSION

There is a belief in Bulgaria that medicinal plants collected at sunrise after Saint John's Eve celebration (24th June) have great potential to cure and improve health. Our study did not reveal striking differences in the phenolic content and antioxidant activity of yarrow and thyme herbs collected in the natural habitats in the Rodopi Mts on 24th June and the same herbs obtained from random Bulgarian producers. Although the yarrow plants from both studied regions showed almost identical total antioxidant activities, their phenolic content differed. Since antioxidant capacity is not coming solely from the phenolics but could be due to the presence of some other phytochemicals (*e.g.* ascorbic acid, tocopherol, pigments, essential oils) or to the synergistic effects among them (SENGUL ET AL. 2009; KRAUJALIS ET AL. 2011), it could be suggested that some other compounds in the plants from Sofia region are capable of antioxidant activity, thus compensating the lower content of phenolics. This means that in yarrow, besides polyphenolics, there are additional metabolites to be explored that might have special beneficial effects on human health.

Our results are in accordance with some previous studies, which demonstrated the effect of the habitat on the antioxidant potential of yarrow. For example, considerable variation in accumulation of phenolic compounds among the flowers of *A. millefolium* L. from different localities was observed (BENETIS ET AL. 2008).

In this study, the total amount of the identified phenolics in yarrow flowers from different populations varied from 13.290 to 27.947 $mg.g^{-1}$. Since the examined populations of *A. millefolium* were located in different regions of Lithuania within habitats with different environmental conditions (*e.g.* in microclimate, soil, ultraviolet radiation), it was concluded that the observed diversity could have a genetic basis, but it may be attributed also to the environmental differences. In Bulgaria, Rogova et al. (2015) performed similar screening of the antioxidant potential of the endemic species *A. thracica* Vel., grown *in vivo*, *in vitro* and *ex vitro* conditions, and reported lack of correlation between the phenolics content and the total antioxidant activity. Most of the studies on different *Achillea* species (including the endemic *A. thracica*) in Bulgaria were focused on their essential oil composition with revealing a dependence of the sesquiterpene lactone profile from the habitat or cultivation conditions (Todorova et al. 2000, 2004, 2007; Yordanova et al. 2017).

The comparison of the phenolics and antioxidant activity of all studied yarrow and thyme plants showed that they were several folds lower than in the Chinese white tea. As in other screens for the antioxidant potential of herbs, our work is an *in vitro* approach and the determined values could differ from those *in vivo* since polyphenols undergo extensive modification during digestion via conjugation in the intestinal cells and liver by sulphation, methylation, and glucuronidation (Setchell et al. 2003). Therefore, for revealing the real antioxidant potential of the investigated herbs, it would be more useful to use more antioxidant methods and to investigate the biological activities of the extracts from medicinal plants which can provide more detailed information about the specific roles of the metabolites (Badarinath et al. 2010; Mekinić et al. 2014). However, our results show the potential of the applied screening of total phenolics content and antioxidant activity as a fast approach to overview the general trend in the antioxidant potential and factors (such as genotype, habitat and storing conditions) that influence it in medicinal plant species used for infusions in the households.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

N.G.G. performed experimental work; G.T.C. and M.K.Z. designed the experiments and interpreted the results; M.K.Z. wrote the manuscript.

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