ANTIOXIDANT ACTIVITY OF MEDICINAL TEA EVIDENCED BY ELECTRON SPIN RESONANCE

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Electron spin resonance (ESR) studies were carried out on 5 types of organic tea cultivated in Romania: *Mentha piperita, Hypericum perforatum, Achillea millefolium, Rhamnus frangula, Calendula officinalis* and the results compared with *commercial* Twinnings *black tea*. In function of tea type, spin centers like Mn^{2+} bounded and unbounded by proteins, Fe^{3+} and Cu^{2+} was evidenced. Dry tea leaves show additional sharp line attributed to semiquinone radical which disappeared in tea drinks. The origin of the spin species in analyzed tea leaves and drinks are defined and discussed. The evaluation of antioxidant capacity of analyzed tea drinks was based on measuring the changes of ESR spectrum of radicals as a result of their interaction with antioxidants. The *Mentha piperita* and *Hypericum perforatum* tea exhibit an important scavenging activity. In the case of *Mentha piperita* the scavenging activity is comparable with that of black tea.

Key words: electron spin resonance, antioxidant activity.

1. INTRODUCTION

The medicinal value of tea for prevention and treatment of many health problems are very well known [1]. Tea drinking could be an important source of some essential minerals such as manganese, which activates numerous enzymes. Current studies show that tea contains specific antioxidants and health promoting ingredients, lowering the risk of several diseases so which are essential to human health [2]. The most studied teas are green (non-fermented) tea, black (fermented) tea and oolong (semi fermented) tea and their pharmacological properties have been discussed [3–10]. But in traditional herbal medicine a wide variety of medicinal tea infusions are used.

In this study we used ESR spectroscopy to analyze some medicinal teas in order to monitor the generation and status of free radicals and spin species and to evidence their antioxidant activity. ESR spectroscopy, due to its capability of detecting free radicals and spin centers, identification of irradiated foodstuffs, chelating properties of antioxidant samples and kinetic physical changes in some food is a powerful tool widely used in medicine and food research [11–14]. Different techniques can be used to study the antioxidant property of tea, among them ESR spectroscopy is the most efficient one for monitoring the chemical radicals and other spin species existing naturally and forming during various reactions in dries tea and tea drinks [15, 16].

2. EXPERIMENTAL

The medicinal teas: *Mentha piperita (MP), Hypericum perforatum (HP), Achillea millefolium (AM), Rhamnus frangula (RF), Calendula officinalis (CO)* were provided from local producers from nord-west region of Romania dried in identical conditions and stored in dark and cool place. The analyzed black tea (BT) was purchase from Twinnings. Diphenylpicrylhydrazyl radical (DPPH) were purchased from Aldrich. The weighed amount (3.5 g) of tea plants was put in hot water (200 ml) for 5 min and filtered out. The tea drinks and the solution resulted after mixing different quantities of DPPH solution at tea drink (100µl:10µl and 500µl:10µl) were placed in ESR flat cell for liquids measurements.

The ESR measurements were performed with a Bruker ELEXSYS 500 spectrometer in X-band (9.52 GHz). The data processing was performed by Bruker X-EPR software. The ESR spectra were recorded using equal quantities of samples.

3. RESULTS AND DISCUSSIONS

The ESR spectra at room temperature of different dried types of medicinal tea are presented in Figure 1.

The ESR spectrum of BT is composed of a sextet lines with a fine splitting value of 70 G, superimposed on a broad envelope at g~2.00, a single sharp line at g ~ 2.002 and weak line at g = 4.32. The BT was the most studied during the last years and in function of origin and species, it was evidence the presence of some elements in high concentration like Mn, Fe, K, Mg, Na, Ca, Al and other elements such as Cu, Zn, Cr, Pb, Cd in low concentrations [2]. By ESR spectroscopy, spin centers like Mn^{2+}, Fe^{3+} can be observed clearly, while other elements such as Cu^{2+} could be see only in particular types of tea [16, 17]. Our spectrum is similar to previously observed spectrum of black tea from various countries [18, 19]. The relative intensities of the components in the spectra may be slightly different

depending on the fermentation processes, storage conditions, age of leaves, geographic region and the climate [20, 21]. The intense and sharp signal (17 G) situated at g ~2.00 indicate that a stable radical of aromatic origin (semiquinone) exists in the sample. The very weak signal situated at g~ 4.32 could be attributed to Fe^{3+}. Since $^{57}Fe^{3+}$ isotope (I = 1/2) has 2.12% natural abundance, the complex will be diluted and the line arising from this complex are weak [16]. The sextet feature is a characteristic ESR-signal of Mn^{2+} (^{55}Mn nucleus, I = 5/2) that is partially unbounded to proteins [22]. If Mn^{2+} is well bounded to protein system, a broad line centered at g~2.00 is observed.

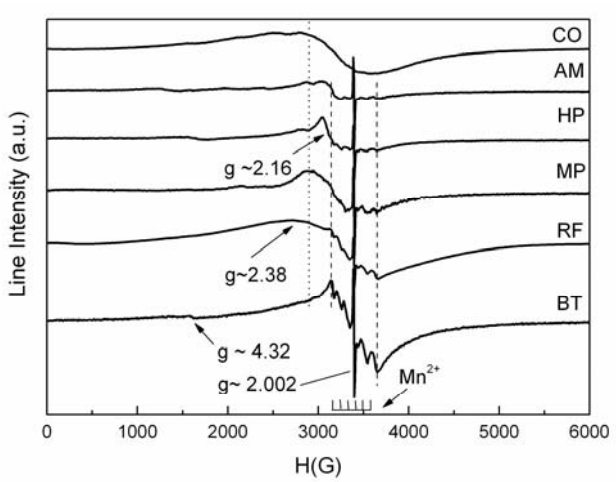

Fig. 1 – ESR spectra of dry tea leaves at room temperature.

A similar behavior was observed for RF plant, but the Mn^{2+} sextet line is not well resolved and the Fe^{3+} absorption line is no more observed. Also, the sharp signal of semiquinone radical decreases in intensity. A supplementary line appears at a field value of about 2800 G (g~2.38) probably due to some asymmetries in the electronic environment of the bonded Mn^{2+}, like a rhombic distortion [23].

The featureless/strong broad signal for CO is typical for Mn^{2+} well bounded to protein system. The ESR line due to Mn^{2+} in rhombic distortions is also present.

The ESR signal for MP, HP and AM is compose of a sharp signal due to semiquinone radical, a sextet lines of Mn^{2+} partially unbounded to proteins, a small shoulder at g~2.38 probably due to Mn^{2+} in rhombic distortions and a signal centered at g~2.16. The signal situated at g~2.16 has a linewidth of about 200 G. A similar signal was observed in *Ginkgo biloba* leaves and was assigned to Cu^{2+} ions [24].

Since tea is prepared in hot water for consumption it has seemed to be necessary to observe what happens to spin species during this preparation process. Fig. 2 shows the recorded ESR spectra at room temperature of the infusions tea.

The ESR spectrum of BT drink compared with black tea leaves shows a sextet lines due to Mn^{2+} unbounded to proteins but the lines intensity is higher. A broad line centered at g~2.00 due to Mn^{2+} bonded by proteins and a supplementary line at g ~2.31 due to Mn^{2+} in distorted sites are also present. The sharp line do to semiquinone radical is no more observed. Compared with BT drink, the ESR spectrum of RF tea contained the same lines but the intensity of the sextet line is higher.

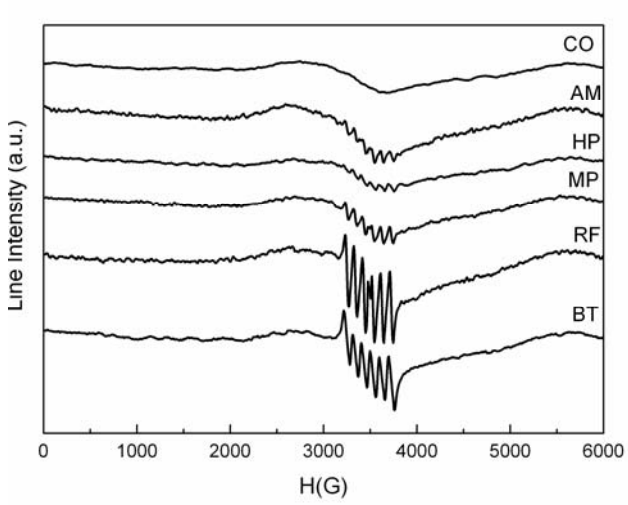

Fig. 2 – ESR spectra of tea drinks at room temperature.

Similar ESR spectra were observed for MP, HP and AM. The sextet lines are better resolved and with higher intensity compared with the spectra for corresponding dry leaves. The intensity of the sextet lines is lower as compared with black tea. The Cu^{2+} ions contribution is no longer observed. The increase of the intensity of sextet lines in tea drinks as compared with leaves sustained the Mn(II)-freeing mechanism observed in another tea drinks [22].

The ESR spectrum of CO tea drink is similar with the spectrum corresponding to dry leaves. Only the contributions of Mn^{2+} well bounded to protein system and Mn^{2+} in rhombic distortions are observed.

The antioxidative properties of tea drinks were also tested. An important mechanism of antioxidant activity is scavenging effect and this effect was evaluated by ESR using stable free radicals DPPH (Fig. 3). The DPPH solutions were mixed with prepared tea drinks and the decline of ESR signal was monitored.

The ESR signal of DPPH is compose of a triplet lines centered at g~2.00 and with a linewidth of 7 G. After addition of tea drinks the ESR signal is reduced in intensity. In Figs. 3a and 3b are presented as an example the DPPH scavenging activity for MP and CO. It is evident that the type of tea drinks influence the

decrease of signal intensity, the evolution in time beeing different. For CO, AM and RF tea a slow decline of ESR signal in time was observed but in the case of MP tea drinks a very important decrease of signal intensity was observed only after 1 min. The MP behavior was also observed for HP and BT. In order to better visualize the radical scavenging activity of these three tea drinks, a higher quantity of DPPH (500 µl) was added to the same quantity of tea drink (10 µl) and after mixing, the resulting solution was measured by ESR (Figs. 3c–d).

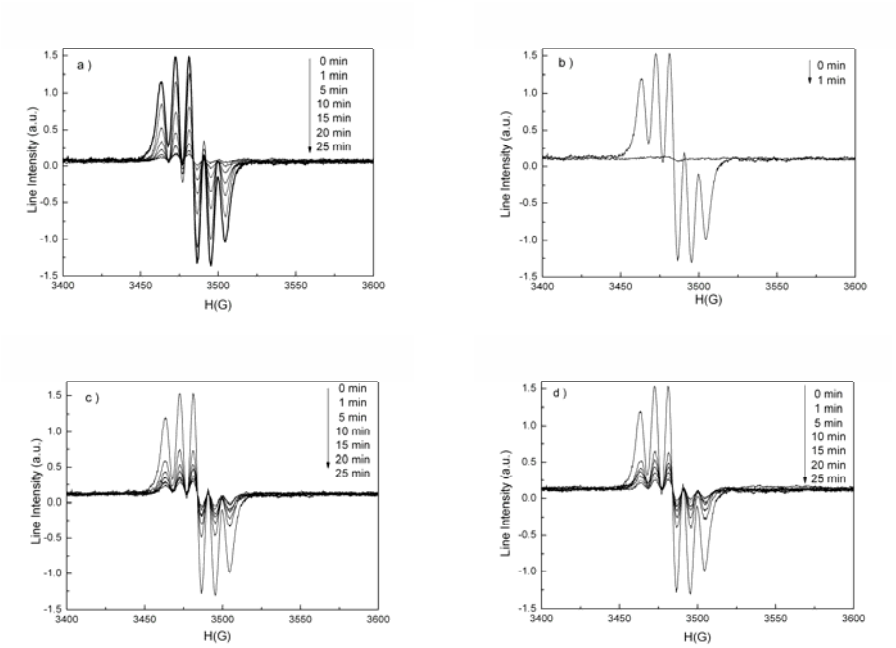

Fig. 3 – Time evolution of ESR spectrum for DPPH radical after mixture with a) CO 100:10; b) MP 100:10; c) BT 500:10 and d) MP 500:10 tea drinks.

The radical scavenging activity was expressed by the ratio $[(I_{DPPH} - I)/I_{DPPH}]$ x 100 (%) where I is the integral intensity of the signal after addition of tea drinks and I_{DPPH} is the integral intensity of DPPH in control solutions. The integral intensity was obtained by double integration of the ESR signal. In the case of mixture of tea drinks with low DPPH quantity (Fig. 4a), the loss of signal was about 98% and 96 % after 1 min for MP and BT, respectively. An important loss of signal was observed for HP, about 95% after 1 min. The same loss of signal was observed in the case of CO and AM but after t = 25 min. The RF tea behavior is different. The ESR signal is reduce 78% in very short time (1 min) and during next 25 min the decrese is not significant.

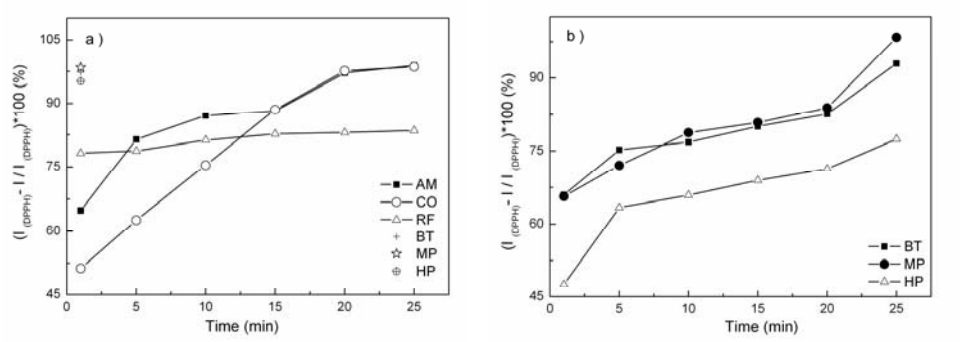

Fig. 4 – The radical scavenging activity of mixture a) 100:10 and b) 500:10 DPPH to tea.

For the analysed mixtures contained a higher quantity of DPPH it can be followed the time evolution of ESR signal for MP, HP and BT (Fig. 4b). The more important radical scavenging activity *i.e.* antioxidant capacity was observed for MP tea. The weakest antioxidant capacity was observed for RF tea.

It was demonstrated previously [25] that the DPPH scavenging activity is influenced by the position, the structure and degree of hydroxylation on the ring structure as well as the electron and hydrogen donating activity of polyphenols, present in tea drinks. The lower antioxidative action of RF tea reflected the presence of different organic acids with lower capability to quench free radical species.

4. CONCLUSIONS

Different medicinal teas from Romania were analyzed by ESR spectroscopy. In all analyzed dry tea leaves the presence of Mn^{2+} in different environments was evidenced. A supplementary ESR signal which probably could be attributed to Cu^{2+} was observed in MP, HP and AM tea. Also a weak contribution of Fe^{3+} was evidenced for BT and HP tea. A stable radical of aromatic origin is present but disappeared in tea drinks. In tea drinks an increase of the intensity of sextet lines attributed to Mn^{2+} ions unbounded by proteins was observed sustaining the Mn(II)-freeing mechanism observed in another tea drinks. An exception constitute the CO tea drinks for which the featureless/strong broad signal typical for Mn^{2+} well bounded to protein system was evidenced.

The antioxidant capacity of tea drinks was evaluated by ESR method using stable free radicals DPPH. The MP tea exhibited the highest antioxidant capacity comparable with black tea. Also, HP tea has an important antioxidant capacity. The weakest antioxidant capacity was observed for RF tea.

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