Comparative Evaluation of Biofunctional Compounds Content from Different Herbal Infusions

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ABSTRACT
Tea is the most important non-alcoholic beverage in the world being appreciated for its stimulant properties and health benefits. The aim of the present study was to evaluate the content in caffeine, phenolic compounds, flavonoids, as well as the antioxidant activity of five different herbal infusions samples in order to compare the amount of these bioactive compounds from traditional Romanian medicinal plants and Chinese tea plants. Green tea, black tea, linden (lime tree), mint and St. John's wort were chosen as materials for the preparation of infusions and laboratory analyses. The caffeine was extracted with dichloromethane and then was quantified by measuring the absorbance of the extract at 260 nm. The quantification of total phenolic compounds was achieved by Folin-Ciocalteu method, while the flavonoids content was determined using a chromogenic system of NaNO$_2$–Al(NO$_3$)$_3$–NaOH based on spectrophotometric method. The antioxidant capacity of each tea sample was assessed by evaluating their radical scavenging activity on DPPH radical. The largest content in antioxidant compounds was found in green (45.90 mg GAE/100 ml), but also in the mint infusion sample (39.30 mg GAE/100 ml), while black tea has registered the highest caffeine content (38.25 mg/100 ml). Following the results obtained it can be stated that all the analysed samples contain remarkable amounts of biologically active compounds essential for the human body health.

Keywords: antioxidant capacity, caffeine, flavonoids, tea, phenolic compounds.

INTRODUCTION
Tea is one of the most common beverages consumed worldwide due to odor, taste and health benefits (Palacios-Morillo et al., 2013). The bioactive components derived from medicinal plants have been traditionally used to prevent and cure many diseases (Kumar et al., 2015). Today, modern medicine has overtaken most of them and has managed to solve many health related problems.

In China, the medicinal effects of tea have a history dating back almost 5000 years. The use of tea in traditional Chinese medicine is well documented and it is suggested that it could be used as a cure for over 200 illnesses (Spices Kerala). The chemical composition of tea may vary depending on a number of factors, such as the conditions in which the plant is grown and how the leaves are processed.

Peppermint (Mentha piperita L.) is one of the most widely consumed single ingredient herbal teas (McKay and Blumberg, 2006). The leaf and oil are used for folk medicine, as flavoring agents, and in cosmetic and pharmaceutical products throughout the world. Also, the world production of peppermint oil is about 8000 tons per year, this being the most extensively used of all the volatile oils (Eccles, 1994; Foster, 1996).

The plant, a hybrid of spearmint (M. spicata L.) and water mint (M. aquatica L.) is indigenous to Europe and widespread in cultivation throughout all regions of de world (Animeshand and Rita,
2011). Mentha piperita, is a natural source of the essential oil menthol, menthone, methyl acetate, volatile oils, tannic acid, terpenes, and vitamin C. The phenolic constituents of the leaves include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin and hesperidin (Balch P.A. and Balch J. F., 2000).

In vitro, peppermint has significant antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and some antiallergenic potential (McKay and Blumberg, 2006). Peppermint oil and menthol have moderate antibacterial effects against both Gram-positive and Gram-negative bacteria (El-Kady et al., 1993). Also, the oil stimulates cold receptors on the skin and dilates blood vessels, causing a sensation of coldness and an analgesic effect (Anonymus, 1999).

Linden is an herb that comes from various species of Tilia, or lime tree. It has been used in European folk medicine for centuries to treat a wide range of health problems. Flowers from two linden species (Tilia cordata and Tilia platyphyllos) were historically used to treat health problems associated with anxiety. Also, it is used in traditional remedies for colds, nasal congestion, throat irritation, headaches, sinus headache and migraine, treatment of palpitations, hypertension, incontinence, hepatitis, colitis, rheumatism, as well as a diuretic and antispasmodic agent (Rodriguez-Fragoso et al., 2008).

The antioxidant content of linden flowers is one of the best qualities this plant has. They contain p-coumaric acid, kaempferol, quercetin constituents and volatile oils terpenoids including citral, citronellal, citronellol, eugenol, and limonene. Antioxidant compounds like quercetin and kaempferol both act as free radical scavengers, eliminating these harmful byproducts of cellular respiration from the system and improving overall health by preventing chronic disease (Newall, 1996).

Extracts of St. John’s wort (Hypericum perforatum) have been used for their medicinal properties since ancient times. Primary production areas for H. perforatum in Europe include Germany, Italy and Romania, and the majority of the harvest goes toward the production of the crude drug (EHG Association). Species of Hypericum contain many bioactive constituents, including proanthocyanins, flavonoids, biflavonoids, xanthones, phenylpropanes, naphthodianthrones that are characterized by their relative hydrophilicity, as well as essential oil components that are more hydrophobic in nature (Crockett, 2010).

Traditionally, St. John’s Wort has a number of different uses including applying it externally to heal wounds and reduce inflammation, or taken internally as an infusion or herbal tea to treat fevers. This herb is widely prescribed for mild to moderate depression and anxiety. Similar to some types of antidepressant medications, St. John’s wort is believed to raise the concentration of serotonin in the brain and enhance its activity (Wheatley, 1998; Henderson et al., 2002).

All of teas made from Camellia Sinensis plant can trace their origins to China. The controlled oxidisation and fermentation methods result in the different types of tea (green, black, and oolong, dark, white and yellow). Green and yellow teas are non-fermented teas and oolong and white teas are called semi-fermented teas, while dark and black teas are fully fermented (Astill et al., 2001; Lee et al., 2008).

Green tea has been enjoyed since ancient times in China and Japan, where it is most frequently drunk but also gained in popularity among Europeans and Americans in recent years for its health-giving properties (Zhao et al., 2014). Also, black tea is among the most widely enjoyed teas in the world especially among the British, who have developed a rich culture around tea.

The health effects associated with the consumption of green or black tea, suggested by recent human studies, are: the reduction of cardiovascular diseases risk and different forms of cancer, reduction of blood pressure, the presence of compounds with anti-Alzheimer’s effect, antibacterial and antiviruses activity, solar ultraviolet protection, bone mineral increase density, source of bio-essential elements (Cabrera et al., 2006; Socaci et al., 2013).

The antioxidant activity and phenolic profile in tea and herbal infusions were studied by Atoui et al., 2015, and their results suggested that black tea and green tea infusions can be major sources of polyphenols that exhibit important antioxidant behaviour.

Due to the increasing interest in tea health benefits, the aim of the present study was to evaluate the content in caffeine, phenolic compounds, flavonoids, as well as the antioxidant...
activity of five different herbal infusions samples in order to compare the amount of these bioactive compounds from traditional Romanian medicinal plants and Chinese tea plants.

MATERIALS AND METHODS
The analysed herbal samples (Green tea, Black tea, Linden, Mint and St. John’s wort) were purchased from local supermarkets (Cluj-Napoca, Romania).

The standard compounds (caffeine, gallic acid, quercetin) and reagents: dichloromethane, 2,2-diphenyl-1-picrylhydrazyl, Folin-Ciocalteu, methanol, aluminium chloride, sodium carbonate, sodium nitrite and sodium hydroxide were purchased from Sigma Aldrich or Merck (Darmstadt, Germany).

Preparation of herbal infusions
The tea brews were prepared by solid-liquid extraction by infusing 2 g of plant leaves in 200 ml of hot deionised water for 5 minutes. After filtration, the extracts obtained were used for total polyphenols, flavonoids, antioxidant activity and caffeine determination.

The caffeine determination
The caffeine was extracted from the infusion samples with dichloromethane and then was quantified by measuring the absorbance of the extract at 260 nm (Jenway, Application Note). An aliquot of 25 ml of each herbal infusion was placed into a separating funnel and 25 ml of dichloromethane was added. The caffeine was extracted by slowly inverting the funnel three times, venting the funnel after each inversion. The dichloromethane layer was removed into a clean flask and the extraction procedure was repeated twice more and the solvent layers combined. A 1000 ppm caffeine standard stock solution was prepared in water. Working standard solutions of 10, 25, 50, 100, 150 and 200 ppm were prepared from the standard stock solution and subjected to the same extraction procedure as the samples. The obtained calibration curve of standards solutions was then used to quantify the caffeine concentration of each herbal infusion sample.

The total phenolic assay
The content of total phenolic compounds was determined following a modified Folin-Ciocalteu method (Singleton et al., 1999). An aliquot of 0.1 ml of infusion was mixed with 6 ml of water and 0.5 ml of Folin-Ciocalteu reagent. After 4 min, 1.5 ml Na₂CO₃ solution (7.5%) was added and the samples were diluted to a final volume of 10 ml with distilled water. After incubation for 120 min at room temperature, in dark, the absorbance was read at 750 nm, using a Shimadzu UV-1700 PharmaSpec spectrophotometer, against the blank, in which the sample was replaced with methanol. Standard curve was performed using different concentration solution of gallic acid and the results were expressed as mg GAE/100 ml infusion (means ± standard deviation).

The total flavonoid assay
The total flavonoid content was determined using a modified aluminium chloride colorimetric method (Zhu H. et al., 2010). An aliquot of 10 ml infusion extract was homogenized with 10 ml methanol and centrifuged at 4000 rpm for 15 min. Then, 1 ml of the supernatant was mixed with 4 ml distilled water, 0.3 ml NaNO₂ (5%) and 0.3 ml AlCl₃ (10%). After 5 min, 2 ml NaOH 1 N and 6.4 ml distilled water were added and the absorbance was measured against the blank (solution without coloration) at 510 nm. Standard curve was performed using different concentrations of quercetin solution and the results were expressed as mg QE/100 ml infusion (means ± standard deviation).

Determination of 2,2-diphenylpicrylhydrazyl radical scavenging capacity (DPPH)
The DPPH scavenging activity assay was performed according to a method reported by Odriozola-Serrano et al., 2008. A volume of 3.9 ml of methanolic DPPH solution was allowed to react in darkness, for 30 minutes with 10 µl of sample and 90 µl of H₂O. The absorbance was measured at 515 nm against methanol. The antioxidant activity was calculated as follows:

\[
\text{% Radical scavenging activity (RSA)} = \left( \frac{A_0 \text{-} A_d}{A_0} \right) \times 100,
\]

where \( A_0 \) was the absorbance of DPPH solution and \( A_d \) the absorbance of the sample.

RESULTS AND DISCUSSION
Nowadays, the content of antioxidant compounds is regarded as a quality indicator of herbal infusions. Therefore, it is desirable to investigate the total polyphenol contents and antioxidant capacities of different teas. The content in total phenols, flavonoids, caffeine and overall antioxidant activity of the analysed herbal infusion samples is presented in table 1.
The highest concentration in phenolic compounds was determined for green tea (45.90 mg GAE/100 ml), closely followed by black and mint tea (39.60 respectively 39.30 mg GAE/100 ml). These data corroborate with those obtained in a previous study by Socaci et al., 2013. Not the same order was observed for flavonoids; John’s wort and mint had the highest content (17.15 respectively 13.24 mg QE/100 ml) followed by the linden sample (8.66 mg QE/100 ml). Both linden and St. John’s wort flowers enclose several flavonoid compounds, especially derivatives of kaempferol and quercetin accompanied by p-coumaric acid (Toker et al., 2001), while peppermint contain large amounts ofroicitrin, luteolin and hesperidin (McKay and Blumberg, 2006). Black and green teas both contain similar amount of flavonoids, however they differ in their chemical structure. Green teas contain more of the simple flavonoids called catechins, while the oxidisation that the leaves undergo to make black tea converts these simple flavonoids to the more complex varieties called theaflavins and thearubigins (Chan et al., 2010). Generally, DPPH radical-scavenging efficiency decreased in the following order: green tea, mint, black tea, St. John’s wort, and linden.

CONCLUSION
The present study evaluated and compared the content in phytochemicals and antioxidant activity of several infusions obtained from Romanian medicinal plants and Chinese teas from local market. Following the results obtained it can be stated that all the analyzed samples contain remarkable amounts of biologically active compounds essential for the human body health. Furthermore, mint infusion samples showed a high content in polyphenolic compounds and flavonoids and an antioxidant activity similar with those of green and black teas.

REFERENCES

Tab.1 The content in total phenols, flavonoids, caffeine and antioxidant activity of infusion samples of some Romanian medicinal plants and Chinese teas

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Herbal infusion samples</th>
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<tbody>
<tr>
<td></td>
<td>Green (GT)</td>
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<tr>
<td>Total phenols (mg GAE/100 ml)</td>
<td>45.90 ± 1.95</td>
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<tr>
<td>Flavonoids (mg QE/100 ml)</td>
<td>4.78 ± 0.30</td>
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<tr>
<td>Caffeine (mg/100 ml)</td>
<td>23.41 ± 0.77</td>
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<tr>
<td>Antioxidant activity (%)</td>
<td>56.99 ± 2.85</td>
</tr>
</tbody>
</table>

(Means ± standard deviation of triplicate analysis)


27. ***European Herb Growers Association (EUROPAM)*** Production of medicinal and aromatic plants in Europe. [Accessed 28.09.2015]. Available online:


