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Mixture Herbal Tea is Oxidant or not?

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Abstract: In recent years, many people have been using various plants and herbal products for preventive or therapeutic purposes. These herbal products, especially herbal teas, have become very popular and have been widely consumed in daily life. We wanted to see whether random, uncontrolled and unmeasured use of these herbal teas, which are consumed without question because they are natural, affect their antioxidant potential. Therefore, in this study, a mixed tea prepared by mixing 10 different herbs, known to be beneficial among the public, in different proportions, sold as "winter tea" in herbalists was used. The antioxidant and oxidant activities of all the plants in this tea were tested separately and in combination, and oxidative stress indexes were calculated. The results showed that no plant included in the winter tea alone had an oxidative stress index as low as the mixture of winter tea. We can say that for the winter tea mixture we chose as the trial material; There is a strong synergistic effect between the herbs in this combination, and the oxidant effect of one was eliminated by the antioxidant effect of the other, resulting in a very safe herbal blend tea. As a result of our study, it has been shown that it is important to determine the oxidative stress state created by different herbal mixtures sold in the market before use. As a result, we cannot say that every herbal tea is harmful or beneficial, and it is not possible to say this for mixture teas. Considering our study result, we can say that winter teas prepared and consumed in this combination do not increase the oxidative stress of the body. Further studies are needed to detail its effects on the body.

Keywords: Medicinal plants, Herbal tea, Oxidative stress, Antioxidant, Winter tea

Introduction

Herbal teas are teas made from parts of plants obtained from natural sources, such as leaves, roots, fruits, which have been used by many civilizations since ancient times for their healing roles. These teas, which are among the most consumed hot drinks among the public, generally contain beneficial substances such as plant extracts, vitamins, minerals, antioxidants and are beneficial for health (Oh et al., 2013). The most common herbal teas are; chamomile, sage, thyme, mint, meadow flower, turmeric, rosehip, rose, cinnamon, lemon balm, linden and it is prepared by one of the methods of decoction and maceration, especially infusion (Kokdil, 2002).

Herbal teas are mainly sold in the market as tea bags, ground or crushed products or dried whole of the plant, but they are also consumed as a mixture tea as well as being offered to the market as a single. Mixed herbal teas are defined as teas in which more than one plant species is used together. These teas, which are marketed as "winter tea", "atom tea", "detox tea", "relaxing relax tea", consist of a mixture of plants with targeted biological activity. For example, "Winter tea" is a Turkish expression and its meaning is generally defined as tea drunk in

cold weather. Winter tea is usually served as hot tea and ingredients such as cloves, walnuts and cinnamon are added to it, especially to warm up in cold weather.

Since all herbal medicines are mixtures of more than one active ingredient, such combinations of many substances clearly increase the likelihood of interactions occurring. However, synergistic effects may occur with the combined use of herbs, as well as antagonistic effects (Junio et al., 2011). In addition, the oxidant properties of these herbal teas, whose antioxidant properties are so prominent, are ignored and there are a limited number of studies dealing with herbal teas from this aspect. Moreover, the positive or negative effects of the use of plants as a single or a mixture on antioxidant and oxidant activities still remain a mystery. From this point of view, in this study, it was aimed to evaluate the oxidant/antioxidant levels and oxidative stress index in order to determine whether the combination of herbs in the blend teas sold as winter tea creates an antagonistic or synergistic interaction.

Method

Supply of Winter Tea and Preparation of Samples

Herbal mixture sold as "Winter Tea" was commercially available from a local herbalist. The mixture was weighed and then separated according to the plants in it. The individual amounts of the separated plants were weighed on sensitive scales and the mixing ratios in winter tea were determined as given in Table 1. By determining the weight of the commercially sold tea bags, the average number of tea bags will be produced from the mixture we have, and how many g of which plant will be in each glass has been determined.

Table 1. The herbs and their amounts in the winter tea mixture

Herb Name	Weight (g)
Chamomillae romanae	6,9746
Hibiscus sabdariffa	5,1643
Rosa sp.	8,9610
Salvia officinalis	1,9055
Cinnamomum zeylanicum	14,2815
Citrus sinensis	4,8918
Rosa canina	31,6920
Tilia cordata	3,2837
Lamium macrodon	2,7826
Curcuma longa	3,4640
Amount	83,4010

Obtaining Tea Extracts

After the proportions in the winter tea were determined, 1.66 g of winter tea was brewed for 5 minutes according to the infusion method in 200 mL mug. Likewise, each herb in the winter tea was weighed 1.66 g and infused for 5 minutes in 200 mL (Figure 1). At the end of 5 minutes, the plants were separated from the water and the herbal teas were left to cool. Thus, 11 samples, including 1 winter tea and 10 herbal teas, were ready.

Figure 1. Extracted winter tea mix and herbal teas

Determination of Total Antioxidant Activity

Commercially available Total Antioxidant Status Assay kit (Rel Assay, Turkey) was used to determine the antioxidant activity of the samples. The basis of this method is based on the fact that the antioxidants in the sample decolorize the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical which is a dark blue-green colored oxidant and give an absorbance change at 660 nm. The amount of change in absorbance is related to the total antioxidant level of the sample. The test is traditionally calibrated with a stable antioxidant standard solution called Trolox equivalent, a vitamin E analogue. Accordingly, 18 µl of tea extracts were added to the wells of the 96-well plate and 300 µl of Reagent 1 in the kit was added to it, and after 30 seconds, absorbance measurement was taken at 660 nm wavelength in the spectrophotometer device. Afterwards, 45 µl of Reagent 2 was added to the wells and incubated at 37 °C for 5 minutes, and then the absorbance was measured again at 660 nm wavelength. Distilled water was used as the negative control, and 1 mmol/L Trolox, the "standard" in the kit content, was used as the positive control. The antioxidant value results were calculated according to the equation given below.

$$\Delta abs = \text{2. Measurement Absorbance} - \text{1. Measurement Absorbance}$$

$$\text{Antioxidant Activity} = \frac{\Delta Abs\ water - \Delta Abs\ sample}{\Delta Abs\ water - \Delta Abs\ Standard}$$

The values obtained as a result of the calculation were interpreted according to the reference values of the kit (Table 2).

Table 2. TAS reference values of the kit

	TAS reference values (mmol Trolox Equiv./L)
>2.0	Very good
1.45 - 2	Normal
1.2 - 1.45	Almost normal
1 - 1.2	Low antioxidant level
<1.2	Very low antioxidant level

Determination of Total Oxidant Activity

Commercially available Total Oxidant Status Assay kit (Rel Assay, Turkey) was used to determine the oxidant activity of the samples. The test is based on the fact that the oxidants in the samples oxidize the iron ion-chelator complex contained in the kit to ferric ion, and the ferric ion formed as a result of this oxidation reaction forms a colored complex with the chromogen. The values obtained as a result of the calculation were interpreted according to the reference values of the kit (Table 3).

$$\Delta abs = \text{2. Measurement Absorbance} - \text{1. Measurement Absorbance}$$

$$\text{Oxidant Activity} = \frac{\Delta Abs\ Sample}{\Delta Abs\ Standard}$$

Table 3. TOS reference values of the kit

	TOS reference values (μmol H_2O_2 Equiv./L)
<5.0	Very good
8 - 5	Normal
12 - 8	High oxidant level
>12.0	Very high oxidant level

Calculation of Oxidative Stress Index

Oxidative stress index (OSI), which is accepted as an indicator of oxidative stress level, is a unitless (AU: arbitrary unit) parameter found by proportioning TOS values to TAS values. In the calculation, the TAS unit (mmol Trolox Equiv./L) was first converted to μmol Trolox Equiv./L, and then the equation given below was used (Deska et al., 2017).

$$OSI = [(TOS, \mu mol\ H_2O_2\ Equiv./L) / (TAS, \mu mol\ Trolox\ Equiv./L) \times 100]$$

Results and Discussion

Total Antioxidant Activity

When the results were evaluated, it was determined that the samples with the highest antioxidant activity had 0.777, 0.667 and 0.662 mmol/L values, respectively, of *L. macrodon*, winter tea and *S. officinalis* extracts. The lowest values were observed in *R. canina*, *C. zeylanicum* and *C. sinensis* with 0.244, 0.269 and 0.274 mmol/L (Figure 2, Table 4).

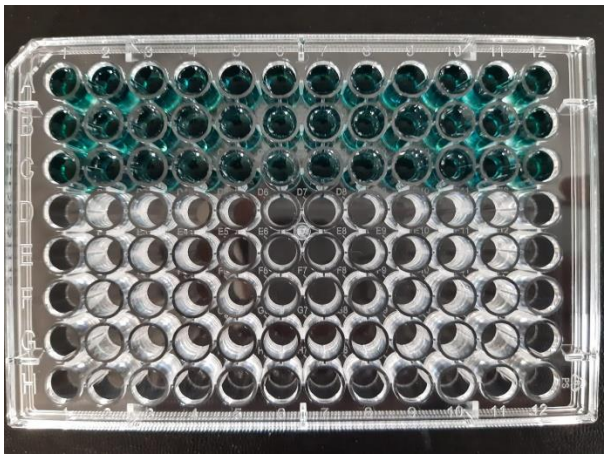

Figure 2. Morphological results of antioxidant activity of herbal teas

Table 4. TAS results of 8.3 mg/mL amounts of winter tea and herbal teas

Herb Name	**TAS Results (mmol/L Trolox)**
Chamomillae romanae	0,303
Hibiscus sabdariffa	0,357
Rosa sp.	0,332
Salvia officinalis	0,662
Cinnamomum zeylanicum	0,269
Citrus sinensis	0,274
Rosa canina	0,244
Tilia cordata	0,405
Lamium macrodon	**0,777**
Curcuma longa	0,296
Winter tea	**0,667**
Positive Control (1mmol/L Trolox)	1

Total Oxidant Activity

According to the oxidant activity results, it is seen that the samples with the lowest oxidant activity are *H. sabdariffa*, *C. longa* and *R. canina* with H_2O_2 values of 4,017, 4,382 and 4,580 µmol/L. These values are followed by 5.606 and 6.651 µmol/L H_2O_2 and *C. sinensis* and winter tea extracts. When we look at the extracts with the highest oxidant activity, we encounter *L. macrodon* with a high value of 31,456 µmol/L H_2O_2, followed by *T. cordata* and *S. officinalis* with 19,331 and 19,080 µmol/L H_2O_2 (Figure 3, Table 5).

Figure 3. Morphological results of oxidant activity of herbal teas

Table 5. TOS results of 8.3 mg/mL amounts of winter tea and herbal teas

Herb Name	**TOS Results (µmol/L H_2O_2)**
Chamomillae romanae	7,921
Hibiscus sabdariffa	**4,017**
Rosa sp.	15,291
Salvia officinalis	19,080
Cinnamomum zeylanicum	8,575
Citrus sinensis	5,606
Rosa canina	4,580
Tilia cordata	19,331
Lamium macrodon	31,456
Curcuma longa	**4,382**
Winter tea	6,651
Positive Control (10 µmol/L H_2O_2)	10

Oxidative Stress Index

According to the oxidative stress index data determined by the ratio of oxidant activity to antioxidant activity, the lowest value was observed in winter tea with 0.997 µmol/L H_2O_2, while it was observed in *T. cordata* with 4.776 µmol/L H_2O_2 (Table 6).

Table 6. OSI values of 8.3 mg/mL of winter tea and herbal teas

Herb Name	**OSI Values (µmol/L H_2O_2)**
Chamomillae romanae	2,616
Hibiscus sabdariffa	1,125
Rosa sp.	4,606
Salvia officinalis	2,883
Cinnamomum zeylanicum	3,182
Citrus sinensis	2,045
Rosa canina	1,873
Tilia cordata	4,775
Lamium macrodon	4,049
Curcuma longa	1,482
Winter tea	**0,997**
Positive Control (10 µmol/L H_2O_2)	1

Discussion

In recent years, many people have been using various plants and herbal products for preventive or therapeutic purposes. The fact that the plants have a high antioxidant value thanks to the active ingredients they contain has a very important role in this. These herbal products, especially herbal teas, have become very popular and are widely consumed in daily life. We wanted to satisfy our curiosity about whether random, uncontrolled and unmeasured uses of these herbal teas, which are consumed without question with the view that everything natural is beneficial, affect the antioxidant potential and make a contribution to this issue. If a plant with a really high antioxidant potential and a plant with a high oxidant potential are in the same mixture, does it reduce the antioxidant effect of tea? Our study results showed that this situation is possible. However, it was observed that the mixture containing plants with high oxidant potential had lower oxidant potential. This gave us the idea that a plant with a high oxidant level may contribute to the immune system with another feature. When used alone, it has been interpreted as the oxidant effect will be eliminated with the mixture and this will increase the contribution of the plant. Moreover, the oxidative stress index of these herbal teas, whose antioxidant activity is trusted, is completely ignored. The fact that the plants that should be consumed by emphasizing their antioxidant activity also have an oxidant level comes to the minds of scientists. However, data on this is very limited. When the existing data are examined, it is seen that many plants also have harmful effects. For example: Long-term use of herbal products with laxative effects such as senna leaf (*Folium sennae*), cassia fruit (*Fructus sennae*), cascara bark (*Cortex rhamni purshianae*) causes diarrhea and disrupts the electrolyte balance as a result of excessive water loss, resulting in hypokalemia. Ginger rhizome (*Rhizoma zingiberis*) inhibits thromboxane synthetase and can change bleeding times. Therefore, caution should be exercised in its use with anticoagulants and should be used under the supervision of a doctor. Animal experiments have shown that eucalyptus essence (*Aetheroleum eucalypti*) and leaf (*Folium eucalypti*) can stimulate liver enzymes and therefore reduce the effects of drugs (Uzun et al., 2014).

However, mixture teas in which herbs are used together are also thought-provoking in terms of benefit and harm comparison (Stermitz et al., 2000). The plants in the mixture can benefit each other by increasing the effectiveness of each other by making an additive effect to each other, and this activity can reach the amount of toxic dose and cause serious side effects that can lead to death. They may not show the expected benefit by dampening the effects instead of increasing each other's effectiveness (Colalto, 2010; Ulrich-Merzenich et al., 2010).

From this point of view, the question that led to the emergence of this project was whether we should consume herbal teas individually or in a mixture. It has been thought whether the mixture teas show the antioxidant properties of all plants together and become a healthier formula than they are individually, or whether their oxidant effects are multiplied and, on the contrary, gaining a harmful dimension to human health.

Therefore, in this study, a mixture of tea, which is sold as "winter tea" in herbalists, was used. There were 10 different plants known to be beneficial among the public in different proportions in this mixture. The plants in this tea were separated and the antioxidant and oxidant activities of all plants were tested separately. At the same time, it was possible to compare the differences according to the single use of the plants by working with the winter tea as a mixture. Then, the oxidative stress index, which is the ratio of oxidant activity to antioxidant activity, was calculated.

When the results were evaluated, considering that the antioxidant and oxidant values were measured for the OSI calculation and the most important thing was the balance between the antioxidants and oxidants in the cell, it was seen that no plant included in the winter tea alone was as close to the balance (approximately 1) as the winter tea mixture. We can say that for the winter tea mixture we chose as the trial material; There is a strong synergistic effect among the 10 plants in this combination, and the oxidant effect of one was eliminated by the antioxidant effect of the other, resulting in a very safe herbal blend tea.

Numerous studies have demonstrated that herbal extracts as a whole and/or multiple herbs in complex formulations offer better efficacies than equivalent doses of individual active ingredients and/or herbs when used alone, highlighting the significance of synergistic action in herbal therapies (Leonard et al., 2002; Scholey & Kennedy, 2002; Zhang et al., 2014). For example, synergistic effects of five commonly used medicinal herbs extracts thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), spearmint (*Mentha spicata*) and peppermint (*Mentha piperita*) were tested in an in vitro study (Yi & Wetzstein, 2011)

However, it should not be forgotten that; The ingredients of each herbal blend tea may differ from each other and the use of the word "safe" for these is dependent on such trials. It is quite wrong to expect that another mixture with different plants or the same plants in different proportions will show the same effect. In this regard, more detailed studies are needed.

Conclusion

As a result, we cannot say that every herbal tea is harmful or beneficial, and it is not possible to say this for blended teas. However, taking into account our study result, consumption of winter tea, which is settled in traditional use, usually prepared in this combination and spread throughout the society, does not increase the oxidative stress of the body. Further studies are needed to detail its effects on the body.

Recommendations

As a result of our study, it is important to determine at least the oxidative stress state created by different herbal mixtures sold in the market before using them. However, it should not be forgotten that they can contribute to the immune system in different ways, not only in terms of antioxidants, but also in terms of their content. It is a situation that must be taken into consideration when preparing the mixture formulations of plants that may have more negative effects than their contribution in terms of oxidative. Because, mixed teas prepared with different herbs can be in a synergistic interaction, as in our study, or they can be in an antagonistic interaction that will negatively affect each other's effects. Finally, we can recommend that such mixed teas should not be consumed unconsciously (without knowing the content interaction, excessive use, etc.), and should be consumed as a supplement rather than a medicine.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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