Article

Systematic analysis of combined antioxidant and membrane-stabilizing properties of several *Lamiaceae* family Kazakhstani plants for potential production of tea beverages

Alibek Ydyrys[1,2*], Nazgul Zhaparkulova[1], Arailym Aralbaeva[3], Aigul Mamataeva[4], Ainur Seilkhan[5], Syraiyl Sayagul[1+], Maira Murzakhmetova[1+]

[1] Al-Farabi Kazakh National University, Faculty of biology and biotechnology, al-Farabi av. 71, 050040, Almaty, Kazakhstan; Nazgul.zhaparkulova@kaznu.kz
[2] Al-Farabi Kazakh National University, Biomedical Research Centre, al-Farabi av. 71, 050040, Almaty, Kazakhstan; alibek.ydyrys@kaznu.kz
[3] Al-Farabi Kazakh National University, Faculty of Medicine and Health Care, al-Farabi av. 71, 050040, Almaty, Kazakhstan; aray3005@mail.ru
[4] Almaty Technological University, Furkat str. 348/4, 050008, Almaty, Kazakhstan; mamataevabbm1976@mail.ru
[5] Abai Kazakh National Pedagogical University, Dostuk 13,050020, Almaty, Kazakhstan; ainura_seilkhan@mail.ru
*Corresponding author: Al-Farabi Kazakh National University, Biomedical Research Centre, Email: ydyrys.alibek@gmail.com; Tel; +77714634007
* Correspondence: Al-Farabi Kazakh National University, Biomedical Research Centre, Email: ydyrys.alibek@gmail.com; Tel; +77714634007
† These authors contributed equally to this work

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Abstract: From the ancient times, beneficial properties of tea beverages were known. Nevertheless, in the deteriorating environmental conditions, causing significant stress to the human organism, interest in enriching these valuable constituents of the human diet has grown. One of the most important compounds that exhibit wide range of biological activities with especially strong antioxidant action are plant polyphenols. In the course of the experiment, dose-dependent effect of rich in polyphenols extracts isolated from the *Lamiaceae* family Kazakhstani plants were studied on the processes of lipid peroxidation and on the degree of erythrocytes hemolysis. Results are presented in the current paper. Accordingly, among the extracts studied, those from sage, thyme and oregano show the most pronounced membrane-stabilizing activity. Antioxidant and antihemolytic properties of green tea and oregano extract mixtures were similar to that of each individual plant extract. Similar results were obtained when the green tea extract was mixed with peppermint, ziziphora and dragonhead extracts, indicating no discernible synergistic interaction. In contrast, when thyme and salvia extracts were mixed with green tea, the mixtures led to subsequent increase in antioxidant potential and membrane-stabilizing effects, again indicating a synergistic interactions between components in these individual plant extracts.

Keywords: Antioxidants; plant extracts; flavonoids; lipid peroxidation; tea beverages

Published: date

1. Introduction

Plants have a large number of important biochemical compounds that are beneficial to cells, tissues and the whole organism. Biological activities of such compounds depend on their chemical structure. Polyphenols and flavonoids constitute one of the major groups of secondary metabolites that occur in staple food plants, where they perform a wide array of biological functions [1, 2]. Despite the differences in their chemical structure and composition, flavonoids are strong natural antioxidants, which neutralize free radicals, prevent from damaging cells and tissues, and consequently prevent occurrence of pathological processes [3].

Anthropogenic pollution of the environment has undesirable effects on human health, and therefore, the adverse influence of environmental factors on health is becoming increasingly important every year [4]. Unfavorable environmental conditions may be the direct cause of human health problems. Almost 12.6 million deaths each year are attributable to unhealthy environments according to the estimates of the World Health Organization (https://www.who.int). Because they are rich in biologically active compounds, medicinal plants are widely used for therapeutic and prophylactic purposes [5, 6].

Polyphenolic compounds, including flavonoids, have a wide range of effects on body cells and systems, produced by plants, which is widely found in fruits and vegetables are thought to protect against ultraviolet radiation e.g. [7], decreasing the risk of cancerogenesis, complication of cardiovascular disorders, diabetes mellitus, etc., which are accompanied by oxidative stress. And Polyphenols are complex antioxidants with increasing public health significance, particularly in the areas of nutrition, epidemiology, and primary, secondary, and tertiary prevention [8]. Therapeutic, corrective action of polyphenols is based on their ability to bind and neutralize free radicals [9-11].

The free radicals are formed in a body during physiological, biochemical processes and play an important role in the transmission of cellular signals, in the synthesis of prostaglandins and cytokinins, and in the utilization and renewal of cellular structures [12]. As a result, antioxidant reserves are depleted, and organisms develop oxidative stress [13]. Increase in lipoperoxidation product levels, is the main cause of most pathologies [14]. The amount of free radical oxidation does not exceed physiological limits in healthy organism, owing to normal functioning of antioxidant protection system, which neutralizes excess quantities of lipoperoxidation products [15].

Tea beverages represent one of the main groups of food products [16]. They can correct any deficiencies in vitamins, microelements and other essential nutrients due to valuable components from fruits and berries in their composition. Tea beverages may include black or green tea as basic components or consist of herbs only, which possess health-improving properties [17].

The aim of our investigation was to study antioxidant and membrane protective properties of some medicinal plant extracts from the family *Lamiaceae*, as well as their content of polyphenolic compounds and flavonoids, to find ways to improve composition of beverages and increase the antioxidant properties of green and black tea.

2. Materials and Methods

2.1. Laboratory animals

Male Wistar rats (220 ± 30 g) were housed under the standard conditions of light and dark cycle with free access to food and water. Blood and livers were taken as described below. The experiments were coordinated and approved by the local ethical commission of the Kazakh Academy of Nutrition, Almaty, Ka-

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zakhstan (control No. 03/145 of 15.09.2015). All experiments were performed according to national and institutional guidelines, which are in line with Guidelines for reporting experiments involving animals: the ARRIVE guidelines and the directive 06 July 2010 [18]. Rats were euthanized by cervical dislocation under isoflurane anesthesia. The livers were isolated, washed, and perfused with chilled saline. Tissue was minced and homogenized (1:10 w/v) in 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA on ice. The homogenate was centrifuged at 10,000 × g at 4°C, for 20 min. The supernatant was further centrifuged at 100,000 ×g, for 60 min, to obtain the microsomal fraction. The pellet (microsomes) was suspended in a buffer containing 10 mM histidine (pH 7.2), 25% (v/v) glycerol, 0.1 mM EDTA and 0.2 mM CaCl2, and was stored at -20°C. The protein content was measured by the Lowry assay using bovine serum albumin as standard [19].

2.2. Preparation of plant extracts

"Greenfield" trademark tea was chosen due to its popularity on Kazakh markets, as well as the species diversity of products. Along with it, medicinal plants belonging to Lamiaceae family (Oregano – *Origanum vulgare*, Ziziphora bungeana – *Ziziphora bungeana*, Dragonhead – *Dracocephalum integrifolium*, Peppermint – *Mentha piperita*, Motherwort – *Leonurus turkestanicus*, Thyme – *Thymus serpyllum*, Sage – *Salvia officinalis*) were used for investigation. Plant materials were purchased in local pharmacy. Tested plants were crushed and powdered with laboratory mill. One g of crushed and powdered dried parts of the tested plants was extracted with 10 mL of 50% (v/v) aqueous ethanol at room temperature, for 20 hours in the dark, as described previously [20]. The mixture was then centrifuged at 20,000 × g for 10 min, and the supernatant was dried at 37°C in a rotary evaporator. Stock solutions of the dried extracts (100 mg) were freshly prepared in 50% ethanol before use in experiments.

2.3. Estimation of total phenolic and flavonoid content

The amount of total phenolics in the extracts was measured using the Folin-Ciocalteu reagent method [15]. 0.5 mL of each extract (1.0 mg/mL) was added into test tubes containing 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 2% sodium carbonate solution, and the tubes were shaken thoroughly. The mixture was incubated at 45°C with intermittent shaking for 15 min. Absorbance was measured at 765 nm using a PD 303 UV-Vis spectrophotometer (Apel, Japan; here and later). Gallic acid was used as standard to obtain a calibration curve (ranging from 0 to 1 mg/mL). The results were expressed in µg gallic acid equivalents (GAE) per mg dry extract.

The total flavonoid contents were determined using a colorimetric assay, with rutin as standard [16]. The 0.5 mL of each extract (1.0 mg/mL) was mixed with 2.0 mL of distilled water and 150 µl of 5% sodium nitrate. After 6 min, 150 µl of 10 % aluminum chloride and 2.0 mL of 1M sodium hydroxide were added to his mixture and the resulting final solution was left at room temperature for 15 min. Then, absorbance of the mixtures was measured at 510 nm. The results were expressed in µg of rutin equivalents (RE) per mg of dry extract. The calibration curve was prepared in the same manner using 0-1.0 mg/mL of rutoside solutions in methanol.

2.4. Estimation of lipid peroxidation in liver microsomes

Lipid peroxidation (LPO) was assessed by measuring malondialdehyde content in the form of thiobarbituric acid-reacting substances (TBARS) by the method of Ohkawa et al. [18]. Briefly, liver microsomes were preincubated with vehicle or test agents in a buffer containing 50 mM KH_2PO_4 (pH 7.2) and 145 mM NaCl at 37°C under constant stirring for 10 min. The basal and 0.02 mM Fe^{2+}/0.5

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Commented [ЫЭ13]: The error has been corrected Stock solutions of the dried extracts were freshly prepared in 50% ethanol (100 mg) before being used in experiments.

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mM ascorbate-induced microsomal LPO was then determined in a reaction mixture containing 0.9 M sodium acetate buffer (pH 3.5), 0.4% SDS and 20 mM thiobarbituric acid after incubation at 95°C for 60 min. After cooling to room temperature, the mixture was extracted by n-butanol: pyridine (15:1, v/v) and centrifuged at 3,000 × g for 5 min. The organic layer was collected and its absorbance was measured at 532 nm. The MDA concentration was expressed as nmol of TBARS per mg of protein [19, 20].

2.5. Isolation of rat erythrocytes

Blood was collected from rats by cardiac puncture under isoflurane anesthesia followed by humane euthanasia. It was centrifuged at 1000 × g, for 10 min, and plasma and white blood cells were removed. Erythrocyte pellets were washed twice with a buffer containing 5 mM Na2HPO4 (pH 7.4) and 150 mM NaCl and were used immediately for osmotic resistance tests [20].

2.6. Estimation of osmotic resistance of erythrocytes

Osmotic resistance of erythrocytes (ORE) was measured as described previously [23]. Isolated erythrocytes were preincubated with vehicle or test agents at 37°C for 10 min, and subjected to a hypotonic solution of NaCl (0.4%) at 37°C for 20 min, followed by centrifugation at 14,000 × g for 10 min. Hemoglobin absorbance was then measured in the supernatant at 540 nm. The extent of hemolysis was calculated as the percentage of total hemolysis caused by 0.1% Na_2CO_3 [20].

2.7. Statistical data analysis

The results were statistically processed using GraphPad Prism 5.01 Program (GraphPad Software, USA). Statistical analysis of data was performed by the methods of descriptive and comparative statistics. The results were represented as mean ± SD of three independent experiments. Relationship between extract concentration and lipid peroxidation was determined along with the hemolysis degree. Pearson correlation coefficient was calculated in accordance with the nonlinear regression equation; registered changes in the indices were considered reliable for $p \leq 0.05$ with the T-criterion. Statistical analysis of the data obtained in the study of plant extract and tea mixtures was produced with application of Student's t-test; registered changes in the indices were considered reliable for $p \leq 0.05$ with the Fisher test.

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Commented [ЫЭ19]: Erythrocytes were preincubated with plant extracts or Green or Black tea at 37 ° C for 10 minutes and then hemolysis was carried out in hypotonic NaCl solution (0.4%) at 37 ° C for 20 minutes, after centrifugation at 14000 xg for 10 minutes was determined. Hemoglobin absorbance was then measured in the supernatant at 540 nm. The extent of hemolysis was calculated as the percentage of total hemolysis caused by 0.1% Na2CO3.

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3. Results

3.1. *Properties of plant extracts*

Comparison of their antioxidant activities showed that the green tea properties are stronger than those of the black tea. It has also been shown that extracts of oregano, thyme and sage have a protective effect on both erythrocyte membranes and microsomal fractions of hepatocytes.

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Table 1. Lipid peroxidation and membrane-stabilizing properties of several *Lamiaceae* family plant extracts (mean ± SD, n=3).

Species	Total polyphenols (μg GAE /mg)	Total flavonoids (μg RE/mg)	Lipid peroxidation IC_{50} (μg/mg protein)	Membrane-stabilizing properties IC_{50} (μg/mL of RBC)
Oregano	374.5±15.2	325.2±23.3	5.5±0.9	75.1±8.1
Ziziphora	401.5±25.6	336.3±42.1	11.5±2.5	194±11.6
Dragonhead	299.4±13.2	157.5±15.3	7.1±1.5	73.5±6.8
Peppermint	137.5±10.2	82.3±7.6	5.8±0.8	>200
Motherwort	305.2±25.3	285.1±10.2	-	>200
Thyme	264.8±9.6	142.3±15.2	3.3±0.7	194±6.5
Sage	251.5±16.8	118.2±8.7	9.2±2.8	75.8±4.8
Tea bush – Camellia sinénsis (green tea)	168.7±8.5	28.7±5.8	9.7±3.1	114.3±9.5
Tea bush – Camellia sinénsis (black tea)	115.3±8.9	18.8±305	14.8±4.5	>200

The 50% inhibitory concentration (IC_{50}) values (μg/mL) were calculated from a log dose concentration-inhibition curve. Total polyphenols and flavonoids concentration are expressed as mean ± SD of triplicate experiments.

Results of the study of the content of polyphenolic compounds, total flavonoids, and IC_{50} for plant extracts are presented in Table 1. By their IC_{50} value, extracts can be placed in order: thyme< oregano< peppermint< dragonhead< ziziphora, with the IC_{50} value for the antioxidant properties of motherwort higher than those of other species included in this study (table 1).

Based on estimation of membrane-stabilizing properties, that are associated with the IC_{50} values, a conclusion can be made about the most significant membrane-stabilizing effects of oregano and sage. Extract of thyme at a concentration of 200 μg/mL inhibited hemolysis by 50%, whereas IC_{50} value of other extracts could not be assessed in tested concentration range.

Based on the results of study of the content of polyphenolic compounds and common flavonoids, plant extracts can be ranked as: thyme> oregano> peppermint> sage> ziziphora> dragonhead> motherwort. The data obtained correlates with the results of studies of antioxidant and membrane stabilizing properties of plant extracts.

3.2. *Influence of herbal extracts of family Lamiaceae*

It is known that raw materials of plant origin have a multitude of substances, and they have beneficial properties for humans [24-26]. Green and black teas are both obtained from the leaves of *Camellia sinensis* that are different by their production technologies. A significant number of studies have been devoted to the studies of antioxidant properties of green and black tea [27-29].

Table 2. Influence of herbal extracts of family *Lamiaceae* on osmotic resistance of erythrocyte membrane. Note: mean ± SD, n=3. The extent of hemolysis was calculated as the percentage of total hemolysis caused by 0.1% Na_2CO_3.

Extract concentration (μg dry substance/mL ES)

Species	0	25	50	100	200
Oregano – *Origanum vulgáre**	100	82.9±3.4	65.7±3.0	40.9±4.8	33.4±2.0
Ziziphora Bunga – *Ziziphora bung*	100	94.5±4.1	79.8±3.5	66.8±3.6	57.3±6.3
Dragonhead – *Dracocephalum integ* n**	100	95.2±2.1	89.4±4.5	71.2±4.9	67.3±4.9
Peppermint – *Mentha piperita****	100	99.5±2.2	97.9±6.2	89.2±4.5	65.4±3.2
Motherwort – *Leonurus turkestanicus****	100	105.6±6.8	101.3±5.6	96.0±5.7	84.9±6.9
Thyme – *Thymus serpyllum**	100	74.4±7.6	58.6±3.9	57.0±5.7	50.1±2.9
Sage – *Salvia officinalis**	100	64.3±3.5	61.7±4.9	42.4±8.4	34.6±2.3
Green tea*	100	68.8±5.6	60.2±6.2	52.1±2.3	45.4±2.7
Black tea*	100	87.5±6.5	78.7±6.8	64.1±3.4	53.9±5.5

In table 2 pearson correlation coefficient (r_{xy}) values were: for oregano – 0.9175, for ziziphora – 0.9677, for dragonhead – 0.9421, for peppermint – 0.9946, for motherwort – 0.9983, for thyme – 0.8856, for sage – 0.9213, for black tea 0.9499, for green tea 0.8956. Statistical significance of Pearson correlation coefficients were: for oregano p=0.028*, for ziziphora p=0.007**, for dragonhead p=0.0166*, for peppermint p=0.0005***, for motherwort p<0.0001***, for thyme p=0.0456*, for sage p=0.0262*, for black tea p=0.0399*, for green tea p=0.0134*.

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Table 3. Effect of herb extracts of family *Lamiaceae* on the level of lipid peroxidation in the liver microsome. Note: mean ± SD, n=3.

Species by common name	Extract concentration (μg dry substance/mg protein)					
	0	2	5	10	15	20
Oregano**	100	77.3±9.6	44.5±4.5	39.0±7.2	20.3±6.5	12.4±2.9
Ziziphora Bunga***	100	84.8±3.9	66.9±5.9	53.9±3.5	39.1±1.6	25.8±5.3
Dragonhead***	100	91.5±3.2	65.1±4.9	49.4±2.5	34.5±2.9	20.3±4.3
Peppermint**	100	78.7±7.3	62.2±3.6	40.3±4.9	24.4±3.2	16.1±3.9
Motherwort	100	124.3±3.3	117.0±4.8	112.3±7.6	110.1±6.4	106.3±11.3
Thyme*	100	68.4±3.1	26.6±3.7	21.6±4.4	8.8±2.9	4.0±1.3
Sage**	100	84.6±4.4	52.2±5.6	29.3±6.2	14.1±3.7	8.5±2.5
Green tea**	100	90.8±6.7	77.3±7.0	48.2±7.6	35.9±5.9	30.9±6.0
Black tea***	100	95.1±5.2	82.5±6.8	65.3±6.4	48.8±6.9	42.8±5.4

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P < 0.01 **
P < 0.001***

In table 3 pearson correlation coefficient (r_{xy}) values were: for oregano – 0.9316, for ziziphora – 0.9811, for dragonhead – 0.9794, for peppermint – 0.9698, for motherwort – 0.3653, for thyme – 0.8746, for sage – 0.9508, for black tea – 0.99, for green tea – 0.9722. Statistical significance of Pearson correlation coefficients were: for oregano p=0.0069 *, for ziziphora p=0.0005***, for dragonhead p=0.0006***, for peppermint p=0.0014**, for motherwort p=0.4765, for thyme p=0.0226*, for sage p=0.036**, for black tea p=0.0002**, for green tea p=0.011**.

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During the experiments, the suspension of erythrocytes was preincubated with extracts of medicinal plants and subjected to a hypoosmotic solution of NaCl. Osmotic resistance was assessed by the degree of hemolysis. As can be seen from the Table 1, in the concentration range of 0-200 μg/mL practically all the extracts led to the dose-dependent decrease in cell hemolysis. These data clearly demonstrated that extracts of oregano, thyme and sage have antihemolytic activity; they reduced the level of hemolysis by 17%, 24.6%, 35.7%, respectively. Ziziphora, dragonhead and motherwort extract showed antihemolytic properties too, reducing hemolysis of erythrocytes by 43-53% at 200 μg/mL concentrations. Similarly, the peppermint extract exhibits hemolytic properties at a low concentration (25 μg/mL), evident from a slight increase in the level of hemolysis of the red blood cells.

Accordingly, among the extracts studied, those from sage, thyme and oregano show the most pronounced membrane-stabilizing activity. The extracts of these plants showed a significant antihemolitic effect at a concentration of 25

μg/mL, while a similar effect of the remaining extracts was manifested at concentrations above 50 μg/mL.

Study of tea extracts effects on osmotic resistance of cell membranes showed that both types of tea significantly decrease erythrocyte hemolysis in hypotonic solution, due to their membrane protective activities. However, protective properties of black tea were less than green tea. In the concentration range of 0-200 μg/mL, dose-dependent decrease in the level of hemolysis was 54.6% for the green tea extract and 45.8% for the black tea extract.

Moreover, the extracts of all plants used in this study exhibit antioxidant properties, inhibiting the formation of LPO products. Our data show that, the extracts of oregano, sage, thyme, dragonhead, ziziphora and peppermint have significant antioxidant properties. We found that they almost completely inhibit the formation of LPO products at all concentrations tested.

In contrast, the extract of the motherwort showed a prooxidant effect over the whole concentration range, as evident from a higher MDA value relative to control.

As for the antioxidant properties of tea extracts, the green tea significantly inhibited LPO processes at a concentration of 10 μg/mL, while the same concentration of the black tea extract inhibited formation of TBARS by 35%, and 20 μg/mL concentration inhibited LPO level around 70% . Further increases in extract concentration led to the complete depression of the free radical oxidation capacity.

3.3. Antioxidant properties and membrane-stabilizing properties of combination extracts of plants and tea.

As noted at a concentration of 20 μg/mL the content of TBA-active products was reduced by almost 60-90%. The results of the study of extracts potentials are presented in Tables 4 and 5.

Table 4. IC 50 values for the effects of combination extracts of plants and tea (mean+SD).

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No.	Sample	IC_{50} (μg/mg protein, mean+SD)		
		Individual extract mean	In combination with black tea	In combination with green tea
1	Oregano	5.5±0.9	8.9±3.5	6.0±0.8
2	Thyme	3.3±0.7	2.75±0.4	4.3±1.1
3	Sage	9.2±2.8	8.2±2.1	7.3±1.6
4	Peppermint	5.8±0.8	7.3±1.8	8.5±2.3
5	Ziziphora	11.5±2.5	7.5±0.9	9.1±3.1
6	Dragonhead	7.1±1.5	10.3±3.5	9.3±3.8

The 50% inhibitory concentration (IC_{50}) values (μg/mL) were calculated from a log dose concentration inhibition curve.

Under the combination of black tea with oregano and peppermint (Table 4) no significant increase in antioxidant and membrane-stabilizing properties has been demonstrated in comparison with the individual plant extracts. Increased antihemolitic effect of black tea in combination with oregano was observed; though the level osmotic resistance for combined extracts was 47% lower than at the action of the single oregano extract.

Table 5. Membrane-stabilizing properties of combination extracts of plants and tea (mean+SD).

The 50% inhibitory concentration (IC_{50}) values (μg/mL) were calculated from a log dose concentration–inhibition curve.

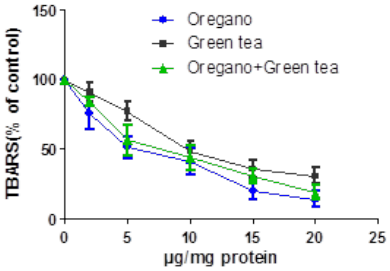
No.	Sample	IC_{50} (μg/mL of RBC, mean+SD)		
		Individual extract mean	In combination with black tea	In combination with green tea
1	Oregano	75.1±8.1	113.0±8.5	79.2±6.8
2	Thyme	194±6.5	178.1±9.8	143.0±7.9

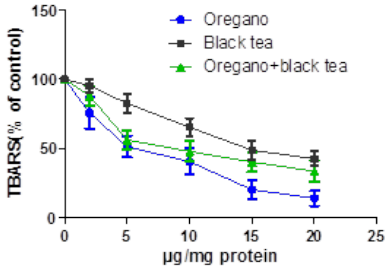
3	Sage	75.8±4.8	70.0±4.8	65.8±5.6
4	Peppermint	>200	>200	118.0±12.3
5	Ziziphora	194±11.6	176.1±8.5	161.0±9.8
6	Dragonhead	>200	> 200	173.4±11.5

Membrane protective effect of the thyme and black tea mixture was greater than that of each individual extract alone. However, no increase in antioxidant potential was observed. The dragonhead and black tea mixture did not lead to significant changes, IC_{50} stayed at its original level. In contrast to the above-mentioned plants, mixing salvia and ziziphora extracts with black tea extract resulted in increased antioxidant and membrane stabilizing effects, which can be explained by a synergic interaction between components in these extracts. Results of the similar studies with mixtures of green tea and other plant extracts are presented in Table 5.

Based on the results of study of the content of polyphenolic compounds and common flavonoids, plant extracts can be ranked as: thyme> oregano> peppermint> sage> ziziphora> dragonhead> motherwort. The data obtained correlates with the results of studies of antioxidant and membrane stabilizing properties of plant extracts. Results on the study of antioxidant effect in the concentration range of 0-20 μg are presented in Figure 1.

(a)





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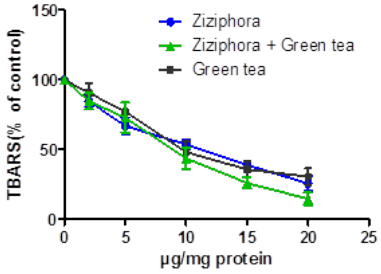

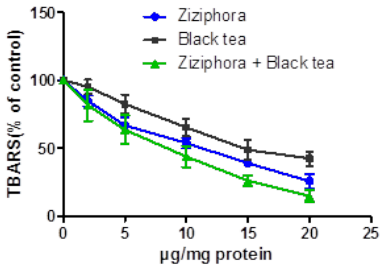

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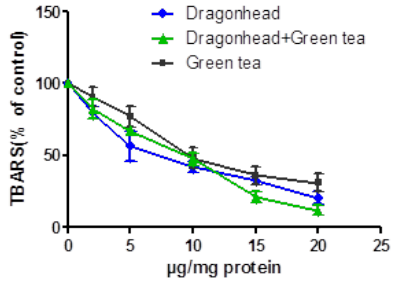
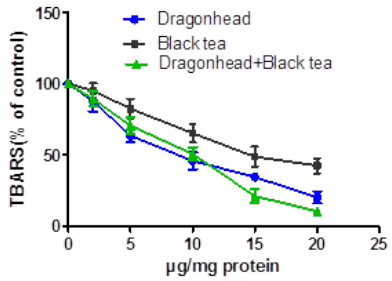
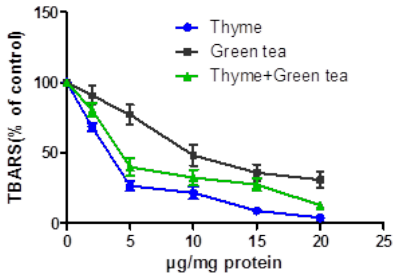
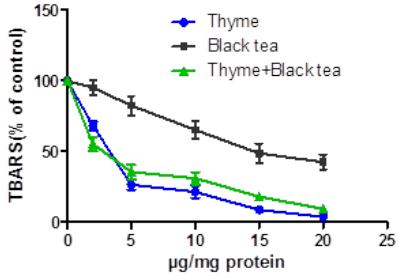

Dragonhead
Dragonhead+Green tea
Green tea
TBARS(% of control)
µg/mg protein

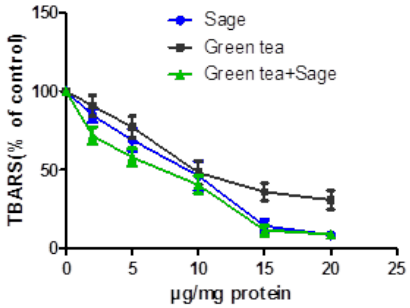

Dragonhead
Black tea
Dragonhead+Black tea
TBARS(% of control)
µg/mg protein

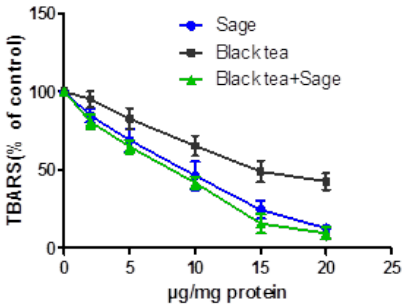
(d)


Thyme
Green tea
Thyme+Green tea
TBARS(% of control)
µg/mg protein

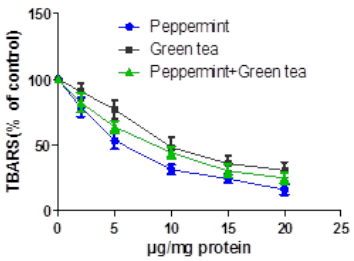

Thyme
Black tea
Thyme+Black tea
TBARS(% of control)
µg/mg protein

(e)


Sage
Green tea
Green tea+Sage
TBARS(% of control)
µg/mg protein


Sage
Black tea
Black tea+Sage
TBARS(% of control)
µg/mg protein

(f)



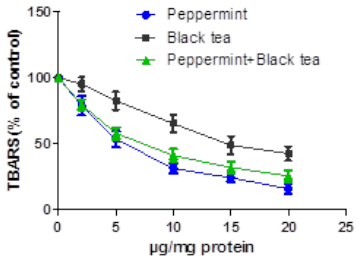


Figure 1. Comparison of single herbal and mixed extracts influence on TBARS level in liver microsomes *in vitro* experiments. Note: on the X-axis: extract concentration, µg/mg, along the Y-axis: level of LPO processes, %; A-oregano, B-ziziphora, C-dragonhead, D-thyme, E-sage, F-peppermint.

Commented [ЫӘ30]: As noted by the reviewer, the old content corrected.

As noted, antioxidant and antihemolytic properties of green tea and oregano extract mixtures were similar to that of each individual plant extract. Similar results were obtained when the green tea extract was mixed with peppermint, ziziphora and dragonhead extracts, indicating no discernible synergistic interaction. In contrast, when thyme and salvia extracts were mixed with green tea, the mixtures led to subsequent increase in antioxidant potential and membrane-stabilizing effects, again indicating a synergistic interactions between components in these individual plant extracts.

4. Discussion

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Data obtained in the study of the effect of plant extracts on the osmotic resistance of erythrocytes and on the processes of lipid peroxidation in microsomal fraction of liver membranes are presented in Tables 2 and 3.

Medicinal plants contain various groups of phytochemical compounds that are natural sources of antioxidants. Large ratio of physiologically active phenolic and polyphenolic compounds in tea suggests the possibility for the important role of tea in prophylactics of several diseases [30-31]. There is evidence from both in vitro and in vivo studies that tea can help to reduce the risk of cardiovascular diseases, certain forms of cancer, and a number of other chronic diseases [32-35]. In order to optimize the antioxidant status of green and black tea and, therefore, enhance their additional protective properties, current studies on the use of plants with significant antioxidant and membrane stabilizing characteristics were performed.

It is well known that black tea loses much of its useful qualities during fermentation processes due to oxidation of the biologically active components. When compared with black tea, green tea has more beneficial effects on living organisms. Both types of tea exert dose-dependent antioxidant effect on membranes of hepatocytes in vitro [36]. Comparison of their antioxidant activities showed that the green tea properties are stronger than those of the black tea. It has also been shown that extracts of oregano, thyme and sage have a protective effect on both erythrocyte membranes and microsomal fractions of hepatocytes.

5. Conclusions

Using plant parts for nourishment, beverage preparation, and disease treatment is a heritage coming from ancient cultures and civilizations. Results of the systematic analysis of antioxidant and membrane-stabilizing properties of Kazakhstani medicinal plants from *Lamiaceae* family joined with common tea beverages demonstrate especially strong synergistic effect of sage, ziziphora and thyme extracts, which deserve consideration for

further study of individual compounds involved in the observed synergy and might be recommended for potential production of tea beverages.

Author Contributions: Conceptualization, N. Aralbaeva and Aigul T. Mamataeva; methodology Maira K. Murzakhmetova; software Syrail Sayagul.; validation, Ainur Seilkhan; formal analysis Arailym N. Aralbaeva; investigation, Nazgul I. Zhaparkulova; resources, Aigul T. Mamataeva; data curation, Alibek Ydyrys and Ainur Seilkhan; writing— original draft preparation, Alibek Ydyrys; writing—review and editing, Maira K. Murzakhmetova; visualization, Maira K. Murzakhmetova; supervision, Maira K. Murzakhmetova. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The experiments were coordinated and approved by the local ethical commission of the Kazakh Academy of Nutrition, Almaty, Kazakhstan (control No. 03/145 of 15.09.2015). All experiments were performed according to national and institutional guidelines, which are in line with Animal Research: Reporting of In Vivo Experiments (ARRIVE 2.0) guidelines and the directive 14 July 2020.

Informed Consent Statement: Not applicable, as the study does not involve any identifiable human biological material.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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