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PRACA ORYGINALNA
ORIGINAL PAPER

Investigation of antioxidant properties and influence on activity of collagenase and elastase of selected raw herbal materials from traditional Eastern medicine

Badanie właściwości antyoksydacyjnych wybranych surowców zielarskich z tradycyjnej medycyny wschodniej oraz ich wpływu na aktywność kolagenazy i elastazy

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ABSTRACT

INTRODUCTION: Traditional Eastern medicine (TEM) is becoming increasingly more popular in highly developed Western countries as an alternative form of supporting health and body care. Many herbs used in this medical practice possess antioxidant, anti-inflammatory and immunomodulatory effects. The skin aging process may progress with age, when collagen and elastin fibers gradually decrease. Excessive exposure to UV radiation, resulting in an increase in the production of free radicals, leads to damage at the molecular level to numerous structures in the body including the acceleration of skin aging.

MATERIAL AND METHODS: The content of polyphenolic compounds (among others: phenolic acids and flavonoids), antioxidant potential (ABTS, DPPH and FRAP assays) as well as the influence on the activity of enzymes, collagenase and elastase, were determined in infusions obtained from *Gynostemma pentaphyllum*, *Tinospora cordifolia*, *Astragalus membranaceus*, *Codonopsis pilosula*, *Asparagus racemosus* and *Ocimum sanctum*.

RESULTS: The highest content of polyphenolic compounds and the strongest antioxidant properties were observed in the infusions obtained from the *O. sanctum* herb, while the greatest ability to inhibit collagenase and elastase was observed in the infusions obtained from the *T. cordifolia* leaves.

CONCLUSIONS: Infusions from the *O. sanctum* herb and *T. cordifolia* leaves may have a potentially beneficial effect on the skin and may be used in anti-aging formulations.

KEYWORDS

antioxidant properties, polyphenols, collagenase, elastase, *Ocimum sanctum*, *Tinospora cordifolia*, *Gynostemma pentaphyllum*, *Astragalus membranaceus*, *Codonopsis pilosula*, *Asparagus racemosus*

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STRESZCZENIE

WPROWADZENIE: Tradycyjna medycyna wschodnia (*traditional Eastern medicine* – TEM) zyskuje coraz większą popularność w wysokorozwiniętych krajach Zachodu jako alternatywna forma wspierania zdrowia i pielęgnacji ciała. Wiele ziół stosowanych w tej praktyce medycznej posiada działanie antyoksydacyjne, przeciwzapalne czy immunomodulujące. Proces starzenia się skóry może postępować wraz z wiekiem, kiedy stopniowo ubywa włókien kolagenowych i elastynowych. Nadmierna ekspozycja na promieniowanie UV, pociągająca za sobą wzrost produkcji wolnych rodników, prowadzi do uszkodzeń na poziomie molekularnym licznych struktur w organizmie i m.in. przyspieszenia starzenia się skóry.

MATERIAŁ I METODY: W naparach otrzymanych z surowców pozyskanych z *Gynostemma pentaphyllum*, *Tinospora cordifolia*, *Astragalus membranaceus*, *Codonopsis pilosula*, *Asparagus racemosus* oraz *Ocimum sanctum* określono zawartość związków polifenolowych (m.in. kwasów fenolowych oraz flawonoidów), potencjał antyoksydacyjny (metodami ABTS, DPPH oraz FRAP), a także wpływ na aktywność enzymów – kolagenazy oraz elastazy.

WYNIKI: Najwyższą zawartość związków polifenolowych oraz najsilniejsze właściwości antyoksydacyjne obserwowano w naparach pozyskanych z ziela *O. sanctum*, natomiast największą zdolność do hamowania kolagenazy i elastazy wykazywały napary pozyskane z liści *T. cordifolia*.

WNIOSKI: Napary z *O. sanctum* i *T. cordifolia* mogą wykazywać potencjalnie korzystny wpływ na skórę, a także znaleźć zastosowanie w formulacjach stosowanych jako produkty przeciwstarzeniowe.

SŁOWA KLUCZOWE

właściwości antyoksydacyjne, polifenole, kolagenaza, elastaza, *Ocimum sanctum*, *Tinospora cordifolia*, *Gynostemma pentaphyllum*, *Astragalus membranaceus*, *Codonopsis pilosula*, *Asparagus racemosus*

INTRODUCTION

Recently, a significant increase in the interest in natural medicine has been observed in highly developed Western countries. Ever more attention is being paid to the plants of traditional Eastern medicine (TEM), which are gaining popularity among people looking for alternative forms of health support. They are being used not only because of their centuries-old tradition in natural healing, but also because of their proven biological activities, such as antioxidant, immune--boosting, anti-aging and metabolism-regulating properties. Their effects are still intensively researched by scientists who are looking for new methods of treatment and to supplement traditional methods of treatment with natural remedies and less invasive methods of taking care of the health and vitality of the body [1,2].

The excessive accumulation of reactive oxygen species (ROS) in the body may cause oxidative stress (OS), which may further result in damage to many structures in the body at the molecular level. The destructive effect of OS on the body's structures makes it one of the main factors contributing to the aging of skin, which, as a natural barrier, is constantly exposed to multiple damaging factors. One of them is the exposure to UV radiation, which directly increases the concentration of ROS, and in turn, activates numerous mechanisms leading to premature aging of the dermis by causing inflammatory reactions and the breakdown of proteins responsible for the proper structure of the tissue [3,4,5,6].

The most important building blocks of the human dermis, responsible for maintaining its proper structure and mechanical properties, are proteins – elastin and collagen. Elastin forms a network of fibers that provide the skin with elasticity and resilience, while collagen is the most extensive protein structure in the dermis layer, giving it resistance to stretching [6,7,8]. The presence of free radicals (FR), generated, among others, as a result of exposure to UV radiation, directly contributes to the overactivity of proteolytic enzymes – elastase and collagenase – responsible for the breakdown of the above-mentioned building proteins. This may result in damage to the structure of the dermis and premature skin aging [6,7].

Among TEM herbs, the following deserve special attention: Gynostemma pentaphyllum, Tinospora cordifolia, Astragalus membranaceus, Codonopsis pilosula, Asparagus racemosus and Ocimum sanctum.

Gynostemma pentaphyllum

G. pentaphyllum, from the *Cucurbitaceae* family, is a folk remedy used to eliminate fatigue, increase endurance and achieve longevity. The active compounds of this plant include triterpenoid saponins (including gypenosides), sterols, flavonoids and polysaccharides [9]. Gypenosides are known to enhance an immune function in oncology patients as well as prevent immunosuppression caused by chemotherapy and radiotherapy, while some even suggest their direct anti-cancer effect [9,10]. *G. pentaphyllum* is a strong cellular antioxidant that may be useful for people affected by diseases associated with the presence of an increased amount of FR [9,10,11].

Tinospora cordifolia

T. cordifolia belongs to the *Menispermaceae* family and is rich in numerous active ingredients, such as

alkaloids, steroids, diterpene compounds (including tinosporone, tinosporic acid and cordifolisides), sesquiterpenoids, polyphenols, aliphatic compounds or polysaccharides [12]. This plant has been a basic component in TEM for centuries, and the current interest in it results from its potential: anti--inflammatory, antioxidant, anti-allergic, immunomodulatory and anti-cancer effects [12,13].

Astragalus membranaceus

A. membranaceus from the *Fabaceae* family is a source of active substances, such as polysaccharides, triterpene saponins, flavonoids or alkaloids [14,15]. There are indications that its polysaccharides have significant anti-aging effects resulting from antioxidant mechanisms [15,16]. It has been proven that A. *membranaceus* effectively inhibits inflammatory reactions by suppressing the release of inflammatory mediators [16]. Moreover, it can inhibit excessive sweating by acting as an antiperspirant and reduce skin inflammation, supporting the healing of boils and abscesses [17].

Codonopsis pilosula

C. pilosula belongs to the *Campanulaceae* family, and the raw material obtained from it is rich in triterpenoid saponins (including tangshenosides), sesquiterpenes (such as atractylenolides), alkaloids, phytosterols, essential oil and polysaccharides [17,18,19,20]. Thanks to its ability to increase the number of erythrocytes and hemoglobin content in the blood, it is used as a strengthening agent for people who are weak, tired and suffering from vitamin and mineral deficiencies. In China, *C. pilosula* root is also used to support anti-cancer treatment and prevent side effects associated with the mentioned therapy, including myelosuppression and immunosuppression [18,19, 20,21].

Asparagus racemosus

A. racemosus from the *Asparagaceae* family is used in TEM as a remedy to improve general well-being by increasing physical strength, improving memory and having anti-aging properties. It is also used in the treatment of infectious diseases and immune disorders [22,23]. Its effects may be due to the presence of anti--inflammatory steroid glycosides (such as shatavarins) and polysaccharides that stimulate the immune system. *A. racemosus* is also rich in flavonoids, alkaloids (including asparagamine and racemosol), vitamins, essential fatty acids and minerals [22,24,25].

Ocimum sanctum

O. sanctum is a plant belonging to the *Lamiaceae* family, whose essential oil is rich in eugenol, carvacrol, linalool, caryophyllene and methyleugenol. It is also a good source of phenolic acids such as: chlorogenic acid, caffeic acid, gallic acid, *p*-coumaric acid, ferulic acid and sinapic acid [26]. This plant is believed to have antioxidant, anti-inflammatory, anti-allergic, immunomodulatory or even anti-cancer properties [27,28,29,30].
Presumably, the antioxidant, regenerating and immunomodulatory properties of TEM herbs could be used in externally applied formulations that would improve the overall condition of the skin. Accordingly, the aim of this research was to investigate the content of selected polyphenolic compounds, antioxidant capacities and collagenase- and elastase-inhibiting abilities from *G. pentaphyllum*, *T. cordifolia*, *A. membranaceus*, *C. pilosula*, *A. racemosus* and *O. sanctum*.

MATERIAL AND METHODS

Chemicals and laboratory equipment

Arnow's reagent, calcium chloride, iron (II) sulfate, iron (III) chloride, hydrochloric acid and sodium acetate were obtained from Chempur, Piekary Śląskie, Poland. Aluminum trichloride hydrate, ascorbic acid (vitamin C; VC) and Folin-Ciocâlteu reagent were procured from Eurochem BGD Sp. z o.o., Tarnów, Poland. Sodium carbonate was derived from Polskie Odczynniki Chemiczne SA, Gliwice, Poland. Rutoside was obtained from Carl Roth GmbH, Karlsruhe, Germany. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), caffeic acid (CA), collagenase (*Clostridium histolyticum*), 2,2--diphenyl-1-picrylhydrazyl (DPPH), N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA), porcine pancreatic elastase, gallic acid (GA), sodium chloride, N-succinyl-Ala-Ala-Ala-*p*-nitroanilide (Suc-$(Ala)_3$--*p*NA), tricine, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and tris(hydroxymethyl)aminomethane (TRIS) were procured from Sigma-Aldrich, St. Louis, MO, USA. Ethyl alcohol 96%, methyl alcohol and sodium hydroxide were purchased from P.P.H. „Stanlab" Sp. z o.o., Lublin, Poland. Epigallocatechin gallate (EGCG) was obtained from TCI, Tokyo, Japan.
Spectrophotometric measurements were made using a Tecan NanoQuant Infinite M 200 Pro microplate reader.

Preparation of plant-derived infusions

The herbal raw materials used in the study were purchased in ground form from an online store PLANTAGO (Figure 1). To obtain aqueous extracts (infusions), they were extracted with distilled water (100°C) and filtered after 30 minutes through membrane filters (0.45 μm pore size). Ten parts of the infusion were obtained from one part of the raw material. Before further determinations were made, each infusion was appropriately diluted according to Table I.

Fig. 1. Ground plant raw materials from traditional Eastern medicine. J – *Gynostemma pentaphyllum*; G – *Tinospora cordifolia*; H – *Astragalus membranaceus*; D – *Codonopsis pilosula*; S – *Asparagus racemosus*; T – *Ocimum sanctum*.

Table I. Herbs of traditional Eastern medicine – raw materials used and their dilutions

Latin name	Traditional name	Abbreviation	Raw material	Polyphenol compounds content		Antioxidant properties – dilutions	Proteolytic enzymes (collagenase and elastase) inhibitory activities
				Phenols and phenolic acids content – dilutions	Flavonoid content – dilutions		
Gynostemma pentaphyllum (Thunb.) Makino	*Jiaogulan*	J	herb	1:10	non-diluted	1:10	non-diluted
Tinospora cordifolia (Willd.) Miers	*Guduchi*	G	leaf	1:4	non-diluted	1:4	non-diluted
Astragalus membranaceus Fisch. ex Bunge	*Huang Qi*	H	root	1:2	non-diluted	1:2	non-diluted
Codonopsis pilosula Nannf.	*Dang shen*	D	root	1:2	non-diluted	1:2	non-diluted
Asparagus racemosus Willd.	*Shatavari*	S	root	1:10	non-diluted	1:10	non-diluted
Ocimum sanctum L.	*Tulsi*	T	herb	1:50	1:10	1:50	non-diluted

Polyphenol compounds content in TEM herbal infusions

Total phenol content

The determination of total phenol content (TPC) was performed based on the modified method of Lunić et al. [31], in which polyphenols react with Folin-Ciocâlteu reagent to form a blue-coloured product. Samples containing series of appropriate dilutions of TEM infusions, as well as a 50% ethanolic solution of gallic acid as the reference compound (concentrations 0––100 μg/mL) were applied to a microtiter plate in the volume of 20.0 μL. 100 μL of 10% aqueous Folin--Ciocâlteu reagent was then added and the microtiter plate was incubated and shaken in the dark for 6 minutes. The next incubation was preceded by the addition of a 7.5% aqueous sodium carbonate solution in a volume of 80 μL. The absorbance of the resulting bluish product at 740 nm was measured spectrophotometrically after 2 hours. TPC was expressed as μg of gallic acid equivalents (GAE) per mL of infusion.

Total phenolic acid content

Total phenolic acid content (TPAC) was evaluated by using a modified method of Lunić et al. [31], where Arnow's reagent in combination with phenolic acids creates an orange-red color of the solution. Series of appropriate dilutions of TEM infusions or a 50% ethanolic solution of caffeic acid (reference compound – concentrations 0–100 μg/mL) were applied to a microtiter plate in the volume of 10.0 μL. Then a mixture of 20 μL of Arnow's reagent, 20 μL of hydrochloric acid (0.1 M), 20 μL of sodium hydroxide (1 M) and 120 μL of distilled water was added to the microtiter plate and briefly shaken. The absorbance of

the resulting orangish-reddish product at 490 nm was immediately measured spectrophotometrically. TPAC was expressed as the μg of caffeic acid equivalents (CAE) per mL of infusion.

Total flavonoid content

Total flavonoid content (TFC) was determined using a modified method of Mihailović et al. [32], based on obtaining an intense yellow color of the solution as a result of the reaction between flavonoids and aluminum trichloride. 100 μL of series of appropriate dilutions of TEM infusions or rutoside solution as the reference compound (concentrations 0–100 μg/mL), were applied to a microtiter plate, respectively. Spectrophotometric measurement of the absorbance of the yellowish product at 415 nm was preceded by an hour's incubation after adding 100 μL of 2% methanolic aluminum trichloride solution to the microtiter plate. TFC was expressed as the μg of rutoside equivalents (RE) per mL of infusion.

Antioxidant properties in TEM herbal infusions

ABTS assay

The scavenging activity of series of appropriate dilutions of TEM infusions was performed by the modified method of Szałabska-Rąpała et al. [33]. An ethanolic ABTS radical cation solution (200 μL) was added to 4 μL of TEM infusions on a microtiter plate or 4 μL of an aqueous solution of the reference compound – VC (concentrations 0–500 μM). After 6 minutes of incubation of the microtiter plate in the dark, discoloration of the tested solutions was observed and the absorbance was measured spectrophotometrically at 734 nm. The decrease of absorption of the ABTS radical caption was expressed as mM of VC equivalents (VCE).

DPPH assay

The ability to decrease the absorption of the DPPH radical by series of appropriate dilutions of TEM infusions was estimated by the modified method of Szałabska-Rąpała et al. [33]. A methanolic-aqueous (80:20 v/v) DPPH solution (180 μL) was added to the 20 μL of TEM infusions on a microtiter plate or 20 μL of an aqueous solution of the reference compound – VC (concentrations 0–500 μM). After 40 minutes of incubation of the microtiter plate in the dark, a color change from purple to yellow of the investigated solutions was observed and the absorbance was measured spectrophotometrically at 515 nm. The scavenging activity was expressed as mM of VCE.

Ferric reducing ability of plasma (FRAP) assay

The capacity of series of appropriate dilutions of TEM infusions to reduce iron in a TPTZ reagent was assessed by the modified method of Benzie and Strain [34]. 300 μL of a freshly obtained reaction mixture (acetate buffer pH 3.6, 10 mM TPTZ in a 40 mM hydrochloric acid solution and a 20 mM ferric chloride solution in the proportion 10:1:1) was added to 10 μL of the TEM infusions on a microtiter plate or 10 μL of an aqueous solution of the reference compound – iron (II) sulfate (concentrations 0–500 μM). After 5 minutes of incubation of the microtiter plate in the dark (37°C), the appearance of a bluish color of the studied solutions was observed and the absorbance was measured spectrophotometrically at 595 nm. The antioxidant property determined using the FRAP method was expressed as mM of iron (II) sulfate equivalents (FeE).

Inhibition of proteolytic enzymes in TEM herbal infusions

Inhibition of collagenase

The inhibitory activity of series of appropriate dilutions of TEM infusions was evaluated by using a modified method of Jakimiuk et al. [35]. Samples containing series of appropriate dilutions of TEM infusions as well as an aqueous solution of EGCG as the reference compound (concentrations 0–500 μM) were applied on a microtiter plate in the volume of 25.0 μL. 25 μL of tricine buffer (tricin, sodium chloride and calcium chloride dissolved in water in appropriate proportions) and 25 μL of 50 mU/mL collagenase in tricine buffer were then added and the microtiter plate was incubated for 20 minutes (37°C). Finally, a collagenase substrate – 0.8 M FALGPA in tricine buffer – was added in the volume of 75 μL. The absorbance at 335 nm was measured spectrophotometrically every 1 minute (T = 10 minutes). The inhibition of collagenase activity for the TEM infusions was calculated according to the equation:

$$\text{inhibition of collagenase activity [\%]} = \left(1 - \frac{\frac{\Delta SA}{\min}}{\frac{\Delta A0}{\min}}\right) \times 100$$

Where:
ΔSA/min – change in absorbance of the tested sample within 1 minute
ΔA0/min – change in absorbance of the negative control (without collagenase) within 1 minute

Inhibition of elastase

The inhibitory activity of series of appropriate dilutions of TEM infusions was evaluated by using a modified method of Chiocchio et al. [36]. Samples containing series of appropriate dilutions of TEM infusions as well as an aqueous solution of EGCG as the reference compound (concentrations 0–500 μM) were applied on a microtiter plate in the volume of 40.0 μL. 40 μL of 0.1 M TRIS buffer pH 8.1 and 80 μL of 50 mU/mL elastase in TRIS buffer were then added and the

microtiter plate was incubated for 30 minutes (30°C). Finally, an elastase substrate – 1 mM Suc-$(Ala)_3$-*p*NA in TRIS buffer – was added in the volume of 80 µL. The absorbance at 740 nm was measured spectrophotometrically every 1 minute (T = 10 minutes). The inhibition of elastase activity for the TEM infusions was calculated according to the equation:

$$\text{inhibition of elastase activity [\%]} = \left(1 - \frac{\frac{\Delta SA}{\text{min}}}{\frac{\Delta A0}{\text{min}}}\right) \times 100$$

Where:
ΔSA/min – change in absorbance of the investigated sample within 1 minute
ΔA0/min – change in absorbance of the negative control (without elastase) within 1 minute

Statistical analysis

The results were presented in graphs as the arithmetic mean ± standard deviation (SD). The statistical assessment was prepared using the one-way analysis of variance (ANOVA) and Fisher's least significant differences *post hoc* test (LSD test). The results were considered statistically significant when $p < 0.05$ as well as bars marked with different letters are statistically significantly different.

RESULTS

Polyphenol compounds content in TEM herbal infusions

The highest contents of TPC and TPAC were determined in the infusion of *O. sanctum*, which were 1715.4 ± 26.7 [µg GAE/mL] and 1586.7 ± 470.7 [µg CAE/mL], respectively and the highest content of TFC was determined in the infusion of *G. pentaphyllum*, amounting to 169.6 ± 3.0 [µg RE/mL]. The content of TPC and TPAC marked in the examined infusions decreased in parallel in the *O. sanctum* > *G. pentaphyllum* > *A. racemosus* > *T. cordifolia* > *A. membranaceus* > *C. pilosula* series. In the case of TFC, its content decreased in *G. pentaphyllum* > *O. sanctum* > *A. racemosus* > *T. cordifolia* > *C. pilosula* > *A. membranaceus* series. The TPC and TPAC contents in the *O. sanctum* infusion and the TFC content in the *G. pentaphyllum* infusion were statistically significantly different from the content of these parameters in the other researched infusions as well as from each other. Interestingly, the second, statistically significantly different result from the others was achieved for TPC by *G. pentaphyllum* with a content of 348.8 ± 5.9 [µg GAE/mL], and for TFC, by *O. sanctum* with a content of 89.6 ± 48.9 [µg RE/mL]. The content of polyphenol compounds in the TEM herbal infusions is presented in Figure 2

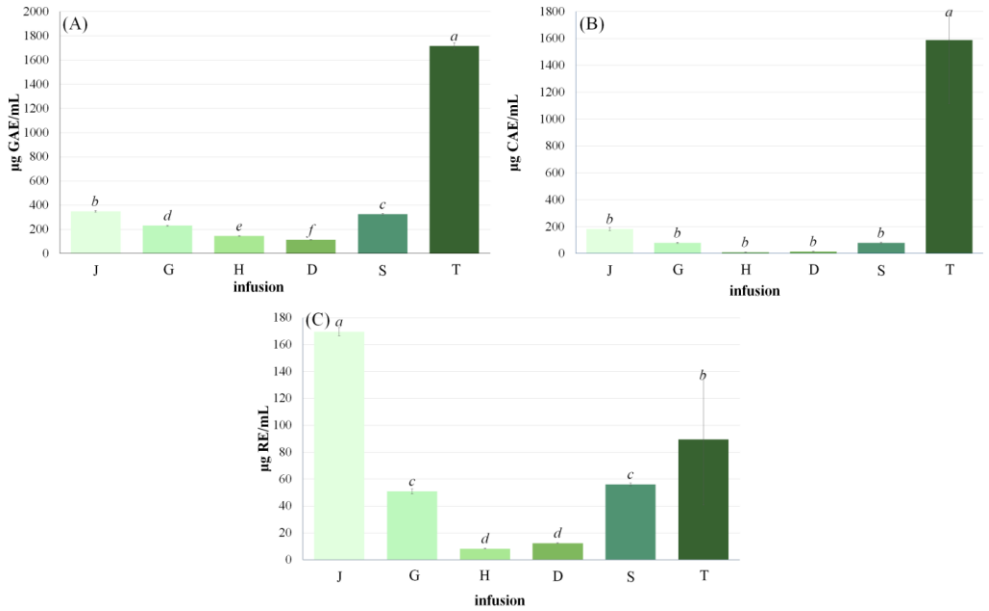

Fig. 2. Polyphenol compounds content in traditional Eastern medicine herbal infusions – total phenol content (A), total phenolic acid content (B) and total flavonoid content (C). J – *G. pentaphyllum* (*Jiaogulan*); G – *T. cordifolia* (*Guduchi*); H – *A. membranaceus* (*Huang Qi*); D – *C. pilosula* (*Dang shen*); S – *A. racemosus* (*Shatavari*); T – *O. sanctum* (*Tulsi*); CAE – caffeic acid equivalents; GAE – gallic acid equivalents; RE – rutoside equivalents. Results are presented as arithmetic mean ± SD. Significances were evaluated with one-way ANOVA followed by Fisher's LSD *post hoc* test. Letter designations above individual bars, if differ from each other, indicate statistical significance ($p < 0.05$).

Antioxidant properties in TEM herbal infusions

In all three methods reflecting the antioxidant properties of the studied infusions, *O. sanctum* occurred to have the best antioxidant activity, which was 14.4 ± 3.9 [mM VCE] for the ABTS assay, 8.2 ± 0.1 [mM VCE] for the DPPH assay and finally 214.9 ± 0.6 [mM FeE] for the FRAP assay. The second highest activity for all the researched parameters was achieved by the *G. pentaphyllum* infusion with the results 3.0 ± 0.2 [mM VCE] for the ABTS assay, 1.4 ± 0.1 [mM VCE] for the DPPH assay and finally 2.6 ± 0.03 [mM FeE] for the FRAP assay. The ABTS, DPPH and FRAP contents in the *O. sanctum* infusion were statistically significantly different from the content of these parameters in the other tested infusions. The *C. pilosula* and *A. membranaceus* infusions had the lowest antioxidant activity in all the determined parameters. The antioxidant properties in the TEM herbal infusions are presented in Figure 3.

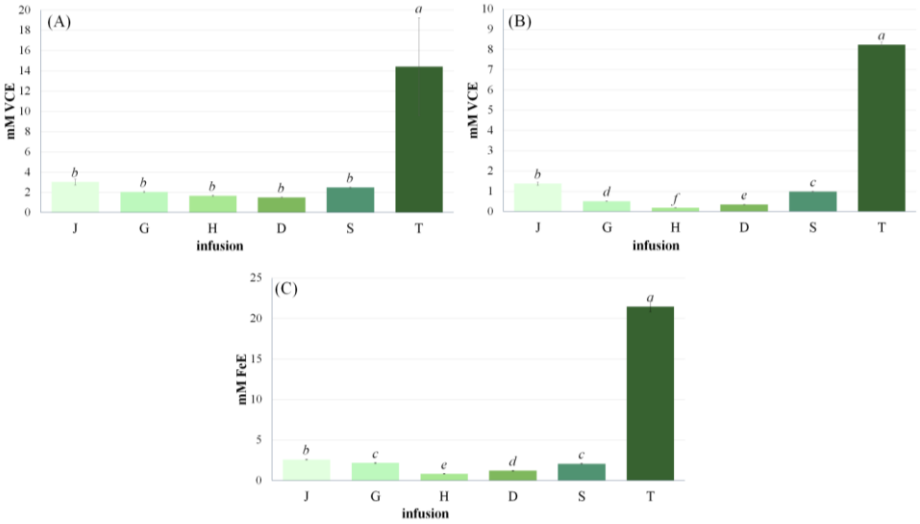

Fig. 3. Antioxidant properties in traditional Eastern medicine herbal infusions – ABTS (A), DPPH (B) and FRAP (C). J – *G. pentaphyllum* (*Jiaogulan*); G – *T. cordifolia* (*Guduchi*); H – *A. membranaceus* (*Huang Qi*); D – *C. pilosula* (*Dang shen*); S – *A. racemosus* (*Shatavari*); T – *O. sanctum* (*Tulsi*); FeE – iron (II) sulfate equivalents; VCE – vitamin C equivalents. Results are presented as arithmetic mean ± SD. Significances were evaluated with one-way ANOVA followed by Fisher's LSD *post hoc* test. Letter designations above individual bars, if differ from each other, indicate statistical significance ($p < 0.05$).

Inhibition of proteolytic enzymes in TEM herbal infusions

The *T. cordifolia* infusion occurred to be the strongest collagenase and elastase inhibitor. Collagenase inhibition by this herb is 54.7 ± 4.4 [%] and elastase 26.0 ± 1.1 [%], while in the case of the first enzyme, this result was not statistically significantly different from the reference compound – EGCG – in the concentration range of 50––100 [μM], and in the case of the second enzyme – from concentrations of 20, 250 and 500 [μM]. The collagenase inhibition by the examined infusions decreased in the *T. cordifolia* > *A. membranaceus* > *A. racemosus* > *C. pilosula* series. The *G. pentaphyllum* and *O. sanctum* infusions occurred to inhibit collagenase activity ineffectively. None of the studied TEM herbal infusions, apart from the *T. cordifolia* infusion, inhibited elastase activity. The inhibition of collagenase and elastase in the TEM herbal infusions is presented in Figure 4.

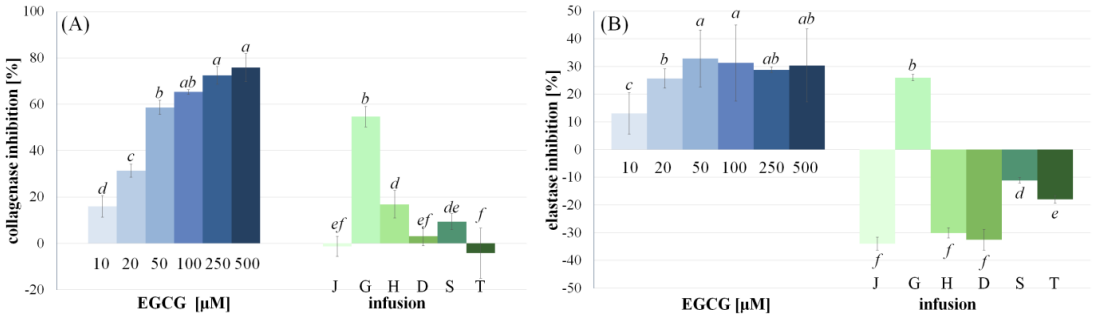

Fig. 4. Inhibition of collagenase (A) and elastase (B) in traditional Eastern medicine herbal infusions. J – *G. pentaphyllum* (*Jiaogulan*); G – *T. cordifolia* (*Guduchi*); H – *A. membranaceus* (*Huang Qi*); D – *C. pilosula* (*Dang shen*); S – *A. racemosus* (*Shatavari*); T – *O. sanctum* (*Tulsi*); EGCG – epigallocatechin gallate. Results are presented as arithmetic mean ± SD. Significances were evaluated with one-way ANOVA followed by Fisher's LSD *post hoc* test. Letter designations above individual bars, if differ from each other, indicate statistical significance ($p < 0.05$).

DISCUSSION

OS can be caused by the accumulation of ROS in the organism, leading to significant damage at the molecular level and premature aging of the body [3,4]. Inhibiting the overproduction of ROS is essential for delaying the aging process of the dermis. Therefore, it seems crucial to search for polyphenolic compounds with antioxidant properties that would counteract the harmful effects of OS.

The highest TPC and TPAC were investigated in the infusion from the *O. sanctum* herb with values at 1715.4 ± 26.7 [μg GAE/mL] for TPC and 1586.7 ± 470.7 [μg CAE/mL] for TPAC. There are studies indicating a high content of phenolic compounds in *O. sanctum*, but owing to differences in the solvents and extraction methods, the results of our study cannot be compared with those of the previously mentioned studies. TPC and TPAC were most often determined in the *O. sanctum* herb using ethanol, methanol, n-hexane, butanol or ethyl acetate as a solvent [26,37,38,39,40,41]. The high content of phenolic compounds in extracts with a polar solvent may be due to the high content of rosmarinic acid as this compound is characterized by good solubility in polar solvents [42]. The occurrence of rosmarinic acid is characteristic of the family *Lamiaceae*, to which *O. sanctum* belongs [42].

The highest TFC was found in the infusion from the *G. pentaphyllum* herb with values at 169.6 ± 3.0 [μg RE/mL], which was confirmed by literature data [43]. Because of the fact that TFC was calculated using RE, it was not possible to refer to other studies. Nevertheless, other studies indicate that the main flavonoids found in *G. pentaphyllum* are: rutoside, 4'-*O*-methylkaempferol-3-*O*-rutinoside, ombuoside, kaempferol-3-*β*-D-*O*-rutinoside, isorhamnetin-3-*O*-β-*D*-rutinoside and quercetin-3--*O*-*β*-D-glucoside [43] and in addition to the above--mentioned flavonoids, it also contains other polyphenolic compounds such as: catechins, procyanidins, tannins or triterpene saponins [9,44].

The ABTS, DPPH and FRAP assays were used to estimate the antioxidant potential of the TEM herbal infusions. The strongest antioxidant properties in each of the assays were obtained by the *O. sanctum* infusion, achieving respectively: 14.4 ± 3.9 [mM VCE], 8.2 ± 0.1 [mM VCE] and 214.9 ± 0.6 [mM FeE]. There is a study of the antioxidant properties of *O. sanctum* using ABTS, DPPH and FRAP assays, but with its n-hexane, acetate or ethanol extracts [45]. Also, it was proven that *O. sanctum* increases the concentration of strong cellular antioxidants: superoxide dismutase and superoxide catalase [27,46], possesses a radioprotective effect (protects against the harmful effects of ionizing radiation) and prevents increasing the concentration of corticosterone – a hormone that is an indicator of increased stress levels [27].

Taking into account the fact that collagenase and elastase are the main enzymes contributing to the breakdown of collagen and elastin, responsible for maintaining the normal structure of cartilage, tendons and dermis, and considering the fact that excessive exposure to UV radiation, triggering the overproduction of FR leads to excessive activity of these enzymes and premature skin aging, it seems beneficial to look for inhibitors of these enzymes [6,7,8]. Collagenase and elastase were most strongly inhibited by the *T. cordifolia* infusion, which achieved a percentage of inhibition of these enzymes of 54.7 ± 4.4 [%] and 26.0 ± 1.1 [%], respectively. To the best of our knowledge, there are no references in the literature about the inhibitory effect of TEM herbal infusions on collagenase activity. However, in the infusion from the stem of *T. cordifolia*, the presence of compounds from the phytoecdysteroid group (such as *β*,5*α*,14*α*-trihydroxyergosta-7,22--dien-6-one) was confirmed. Importantly, ergosterol derivatives can inhibit human neutrophil elastase [47].

CONCLUSIONS

Our results indicate that the strongest antioxidant activity is observed in the infusion of *O. sanctum*, and the strongest inhibitor of collagenase and elastase is the infusion of *T. cordifolia*. Those infusions may have a beneficial effect on the skin and can potentially be used as ingredients of anti--aging products used externally to improve the condition of the skin. Further confirmation of the results obtained in this study is required in *in vivo* models.

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Author's contribution

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