Vol.67: e24220661, 2024
https://doi.org/10.1590/1678-4324-2024220661
ISSN 1678-4324 Online Edition

BABT
Brazilian Archives of Biology and Technology

Article - Human and Animal Health

Influence of Teas on Phospholipase A_2 and Protease Activity in the Context of Blood Hemostasis-Related Processes

Mateus Santos Carapiá[1]
https://orcid.org/0000-0002-7523-1060

Daniela Aparecida Oliveira[1]
https://orcid.org/0000-0002-3576-775X

Marcus Vinicius Cardoso Trento[1]
https://orcid.org/0000-0001-8463-9052

Silvana Marcussi[1]
https://orcid.org/0000-0002-4674-6911

Tatiane Silva de Abreu[1]
https://orcid.org/0000-0002-3809-5442

Pedro Henrique Souza Cesar[1]
https://orcid.org/0000-0003-3271-7797

Mariana Aparecida Braga[1*]
https://orcid.org/0000-0003-0758-3993

[1]Universidade Federal de Lavras, Departamento de Química, Lavras, Minas Gerais, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jane Manfron Budel

Received: 25-Aug-2022; Accepted: 23-Aug-2023

*Correspondence: marybraga07@yahoo.com.br; Tel.: 55-x35-38291893 (M.A.B.).

HIGHLIGHTS

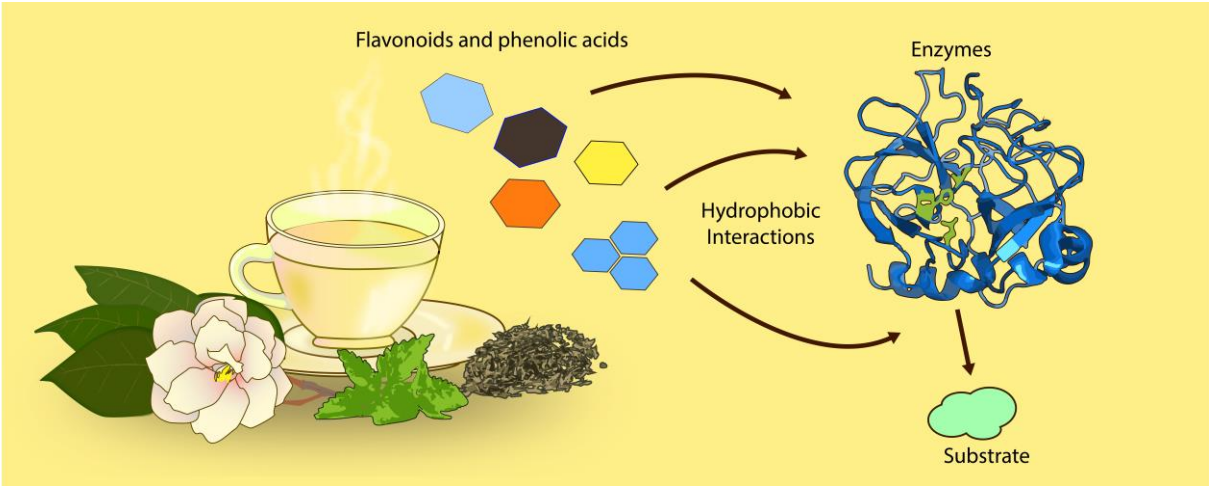
- Effect of teas on enzyme activities.
- Inhibiting hemorrhagic proteases.
- Consumption of herbal infusions could result in benefits to human health.

Abstract: Tea is identified as the second most consumed drink in the world, and its frequent intake is related to several benefits to human health, considering its antimutagenic, anticarcinogenic, antimicrobial, anti-inflammatory, antihistamine, diuretic, calming, and above all, antioxidant effects. These effects are often associated with the action of the phenolic compounds contained in these infusions. In Brazil, among the most consumed infusions are the teas of chamomile (*Matricaria chamomilla* L.), lemongrass (*Cymbopogon citratus* (D.C.) Stapf), lemon balm (*Melissa officinalis* L.), anise (*Pimpinella anisum* L.), yerba mate (*Ilex paraguariensis* A. St.-Hil), peppermint (*Mentha piperita* L.), and green/black tea (*Camellia sinensis* L.). Therefore, said popularity is the reason why the mentioned species were chosen to be evaluated on inflammatory enzymes. The activity of phospholipases A_2 was reduced by more than 25% after treatment with black tea and yerba mate. The most significant inhibition of protease activity was observed after incubation with black tea (40.74%), green tea (31.48%) and yerba mate (25.93%). Infusions of black and green tea reduced hemolysis in semisolid and liquid media, and for the latter, reductions of up to 50% of hemolytic activity were observed, indicating an anti-inflammatory potential of the samples. Plasma incubations with green tea, black tea and lemon balm and subsequent addition of venom (1:10 ratio; tea:PBS, v:v) prolonged the coagulation time of citrated plasma by approximately twice compared to the positive control. All controls with pure tea had a thrombolytic character, in higher proportions than the venom control, especially chamomile (273.55% dissolution). Phenolic compounds derived from phenolic acids, flavonoids,

and tannins are identified as the main agents that promote the biological effects observed in this study. This is mainly due to their anti-catalytic properties exerted on inflammatory enzymes and as chelating agents of enzymatic co-factors. The evaluated teas showed potential for nutraceutical use, thus pointing to the possibility of use as an adjuvant in the treatment of diseases linked to hemostasis.

Keywords: medicinal plants; functional foods; enzymatic inhibitors; toxins as tools.

GRAPHICAL ABSTRACT



INTRODUCTION

Tea has numerous health benefits and is the second most consumed beverage in the world, surpassed only by water [1]. The high consumption of infusions by different populations over the years is related to their antimutagenic, anticarcinogenic, antimicrobial, anti-inflammatory and, mainly, antioxidant effects [2].

The benefits of teas to the body are usually associated with the antioxidant activity of different phenolic compounds, especially flavonoids, present in these infusions [3-5]. Antioxidants are substances that have the ability to inhibit and/or decrease the action of oxidizing compounds and free radicals; in addition, they can act by chelating metal ions [6].

Phenolic compounds, also called polyphenols, are part of a class of substances derived from the shikimic acid and acetate-malonate pathways and occur in a wide variety of structures that have at least one aromatic ring with one or more hydroxyl groups. The antioxidant activity of polyphenols is mainly attributed to the presence of hydroxyl groups in their structure [7]. Phenolic compounds can be grouped into 3 major groups: flavonoids (anthocyanins, flavonols, isoflavones and flavones), tannins and phenolic acids [2].

Among phenolic compounds, flavonoids are the main targets of medical and scientific interest, especially for their anti-inflammatory and hypocholesterolemic properties, which are related to the ability of these compounds to inhibit specific enzymes [8]. Phenolic compounds with antioxidant activity are indicated as important agents in delaying aging as well as in the prevention of degenerative, cardiovascular and brain diseases [9]. In addition to antioxidant activity, these compounds perform other functions in the body, acting as antiatherogens and vasodilators, participating as modulators of enzymatic pathways and gene expression and contributing to improving the functions of cell membranes and receptors [10].

The consumption of beverages or supplements rich in polyphenols has been recommended to reduce the intestinal absorption of iron, since these natural compounds can form stable complexes with cationic metals, and thus reducing their bioavailability [2, 3]. The effects of the food matrix on the bioavailability of phenolic compounds are not scientifically clarified. However, some previously described interactions between phenolics and proteins present in foods indicate the chelation and/or complexation of these compounds with ions that act as enzymatic co-factors [2, 5, 10].

Snake venoms have different enzymes in their composition (e.g., metalloproteases, serine proteases, and phospholipases A_2). These enzymes have structural and functional similarities to human ones, and as such, they can affect physiological processes by altering the inflammatory and immune responses and the hemostasis [11]. Therefore, using them as tools to evaluate possible interactions of natural compounds with different enzymes is acceptable. In the context of the scientific exploration of nutraceutical products, teas stand out for having a composition rich in molecules that act in the prevention and as adjuvants in the

treatment of various diseases of inflammatory origin and development as well as being regulated as foods that can be easily recommended or prescribed, without legal restrictions. Thus, in this study, the main infusions consumed in Brazil (chamomile, lemongrass, lemon balm, anise, yerba mate, peppermint and green/black tea) were characterized in pharmacological and toxicity assays to evaluate their effects on the activity of PLA_2 and proteases (using snake venoms as the study material), aiming to increase the knowledge about the nutraceutical potential of these teas.

MATERIAL AND METHODS

Tea samples and preparation

The plant species chamomile (*M. chamomilla*), lemongrass (*C. citratus*), lemon balm (*M. officinalis*), anise (*P. anisum*), yerba mate (*I. paraguariensis*), peppermint (*M. piperita*) and green/black tea (*C. sinensis*) were purchased from local stores in the municipality of Lavras, Minas Gerais, Brazil. The teas were prepared using 3 sachets (2 g of leaves and/or dried flowers, each) that remained under infusion in phosphate buffered saline (PBS), freshly boiled. The samples were prepared in PBS (pH 7.4) to simulate the physiological pH during the tests, thus guaranteeing the cellular integrity of red blood cells and enzymes used. The content used for direct evaluation in the tests was stored at -20 °C during the study.

The proportions used were 1:0 (v: v – tea: PBS), which means 100% volume of tea and no PBS; 1:1 (v: v - tea: PBS), which is a dilution of 50% tea and 50% PBS; and 1:2 (v: v - tea: PBS), which represents a dilution of 33.33% of tea in 66.66% of PBS.

Human blood and plasma samples

The blood used for the tests was obtained from 10 healthy volunteers of both sexes aged between 20 and 40 years who reported not having used medication for a period of 30 days before blood collection. A volume of 10 mL of blood per volunteer was collected by venipuncture into tubes containing citrate, for coagulation activity assays, containing heparin, for anti-inflammatory and hemolytic activity assays, or without anticoagulant, for thrombolytic activity assays. All experiments were performed according to protocols previously approved by the Human Research Ethics Committee of the Federal University of Lavras (Universidade Federal de Lavras - UFLA) under CAAE registration n. 10587519.1.0000.5148.

Snake venom

For the assays, *Bothrops moojeni* Hoge venom commercially purchased from Bioagents Serpentarium (Batatais, São Paulo) was used. The venom was weighed (10 mg) and dissolved in 1 mL of PBS (pH 7.4) for the assays.

Phospholipase activity

Phospholipase activity was assessed as described by Gutiérrez and coauthors [12]. Phospholipase A_2 inhibition assays were performed using *B. moojeni* venom (30 µg), which was previously incubated with the infusions for 30 minutes at 37 °C. The evaluation of phospholipase activity was performed on agar gel prepared with 0.01 mol L^{-1} $CaCl_2$, egg yolk (1:3, v:v; phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine), PBS (pH 7.4), 1% bacteriological agar and 0.005% sodium azide, pouring the medium at 45-50 °C into Petri dishes. After gel solidification, the treatments were applied in a final volume of 30 µL in 0.5-cm diameter holes. The plates were kept in a cell culture chamber for 18 hours followed by measurement of the halos. The mean values obtained for the controls containing only phospholipases were considered 100% activity. Controls for enzyme inhibition were performed using prednisolone (25 $\mu g.mL^{-1}$, 50 $\mu g.mL^{-1}$, and 75 $\mu g.mL^{-1}$).

Anti-inflammatory activity assessed by erythrocyte membrane stability at 54 °C

Human peripheral blood (10 mL) was collected into tubes containing heparin and immediately centrifuged at 3600 x *g* for 5 minutes, after which the plasma was removed. An aliquot of the platelet-rich erythrocyte concentrate was used to prepare a cell suspension with 2% hematocrit in PBS, pH ~7.4 (v:v). For the evaluation of each treatment, 1.2 mL of a 2% erythrocyte solution were used. Two treatments were prepared in a final volume of 200 µL - the first contained 100 µL of the sample and 100 µL of PBS, and the second had only the sample (green/black tea (*C. sinensis*)). The positive controls were prepared with nimesulide and prednisolone (25, 50 and 75 $\mu g.mL^{-1}$). Negative controls (used as a blank for readings in the

spectrophotometer) were prepared exclusively with PBS (pH 7.4), receiving no additional treatment. Incubation was performed at 37 °C for 30 minutes for extracts in liquid form. After incubation, the tubes containing the treatments were placed in a thermostatic bath at 54 °C for 20 minutes. Then, the incubates were centrifuged at 3600 x *g* for 5 minutes, and the supernatant was read in a spectrophotometer at 540 nm [13-15].

Cytotoxic activity on human erythrocytes

For the cytotoxicity assay using human erythrocytes, the same method described in section 2.4 [12] was used, but phospholipids were replaced with a concentrate of human erythrocytes at the same ratio. To obtain erythrocytes, 10 mL of human blood was collected in the presence of anticoagulant, mixed with the same volume of saline solution (2 mM NaH_2PO_4; 3 mM Na_2HPO_4; 154 mM NaCl; pH 7.4) and centrifuged at 700 rpm (Fanem Baby® I Model 206 BL) for 10 minutes. The plasma was removed, and the erythrocytes were suspended in 5 mmol L-1 PBS, pH 7.4, and centrifuged under the same conditions; this step was repeated twice as described by Preté and coauthors [16]. The inhibition of hemolytic activity was evaluated using *B. moojeni* venom (30 µg) previously incubated with the infusions for 30 minutes at 37 °C in a final reaction volume of 30 µL per sample/per well.

The phospholipase activity and cytotoxic activity on erythrocytes were evaluated by measuring (mm) the translucent halo formed around the holes in the gels where the samples were applied; the results are expressed as a percentage considering the mean of the controls containing only venom as 100% activity.

Proteolytic activity on casein

For the evaluation of proteolytic activity, the method described in sections 2.3 and 2.6 [12] was used, but phospholipids were replaced with casein solution, using this substrate at the concentration reported by Wang and coauthors [17] for caseinolytic tests in liquid medium. Casein solution (5 mg mL^{-1} in 50 mM Tris-HCl buffer (pH 8.0)) was used to prepare the gels.

B. moojeni venom proteases (10 µg) and infusions were preincubated in a final reaction volume of 30 µL per sample for 30 minutes at 37 °C and then applied to the holes made in the gel, followed by incubation for 18 hours at 37 °C in a cell culture chamber. Controls containing only proteases were also evaluated.

The gel was stained with 1% black starch solution and destained in 10% acetic acid solution, enabling the quantification of proteolytic activity by measuring the diameters of the translucent halos formed around the holes. The results are expressed as percentages, where the mean of the controls containing only venom was considered 100% proteolytic activity.

Fibrinogenolytic activity

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli [18], was used to evaluate fibrinogenolytic activity. Protease inhibition assays were performed after preincubation of *B. moojeni* venom (60 µg) with the infusions at a final reaction volume of 30 µL per sample, and half of these samples were incubated for 30 minutes at 37 °C, followed by the addition of fibrinogen and subsequent incubation for 2 hours at the same temperature. Controls containing only fibrinogen and venom were also performed.

The samples were analyzed in a 12% polyacrylamide gel (w: v), allowing the observation of the α, β and γ chains of the control fibrinogen as well as the presence of fibrinopeptides in the samples in which there was proteolysis.

Coagulant activity

The method for evaluating the coagulation time of citrated human plasma was performed as described by Rodrigues and coauthors [19]. The herbal infusions were preincubated with *B. moojeni* venom at a final reaction volume of 30 µL per sample for 30 minutes at 37 °C. The incubates were then added to tubes containing citrated plasma (200 µL), stabilized in a heating bath at the same temperature, and immediately timed until the formation of a rigid clot. Controls containing only venom were also performed. The assays were also performed with prior incubation of the infusions with citrated plasma and the subsequent addition of venom. Thus, possible interactions with proteases or plasma constituents could be evaluated. The minimum coagulant dose was previously defined, i.e., the minimum amount of protease capable of inducing plasma coagulation at an interval between 1 minute and 1 minute and 25 seconds [20].

Activity on blood thrombi

The thrombolytic activity was evaluated on human blood clots formed *in vitro*. The clots were incubated for 24 hours at 37 °C with samples containing only *B. moojeni* venom (30 µg), PBS, infusions or venom

preincubated (30 minutes at 37 °C) with tea infusions at a final reaction volume of 30 µL per sample/per well [21]. The thrombolytic activity was estimated by measuring the volume of liquid released by each thrombus. The mean volume obtained in the controls performed with the venom was considered 100% activity.

Statistical analysis

The results are presented as the mean of triplicates ± standard deviation. The data were subjected to analysis of variance, and the means were compared using the Scott-Knott test ($p<0.05$) with the aid of the statistical program R [22].

RESULTS AND DISCUSSION

Phenolic compounds are important inhibitors of snake venom enzymes and, consequently, of the toxic and/or pharmacological effects associated with the activity of these enzymes [23, 24]. The anti-inflammatory effect of flavonoids is mainly associated with interactions with enzymatic systems, the inhibition of arachidonic acid metabolism [25, 26] and the removal of reactive oxygen species, which are converted into stable resonance hybrids and, subsequently, into quinone [27].

Phospholipase activity

PLA_2 inhibition percentages greater than 25% were observed for the black tea (27.54%) and yerba mate (29.15%) infusions. Lower inhibition percentages were observed for the lemongrass (17.87%), green tea (19.48%), peppermint (21.10%), chamomile (14.65%), anise (13.04%), and lemon balm (6.60%) infusions (Figure 1). The relative standard deviations (all <4%) indicated agreement between the data obtained in the replicates; thus, at the evaluated experimental conditions, the inhibition of PLA_2s of *B. moojeni* was more effective after incubation with the black tea and yerba mate infusions. Controls were prepared with the anti-inflammatory drugs prednisolone and dexamethasone at a concentration of 1 $\mu g.\mu L^{-1}$, obtaining maximum inhibition percentages of 13.04% and 19.48%, respectively, values lower than those observed in most samples containing herbal infusions.

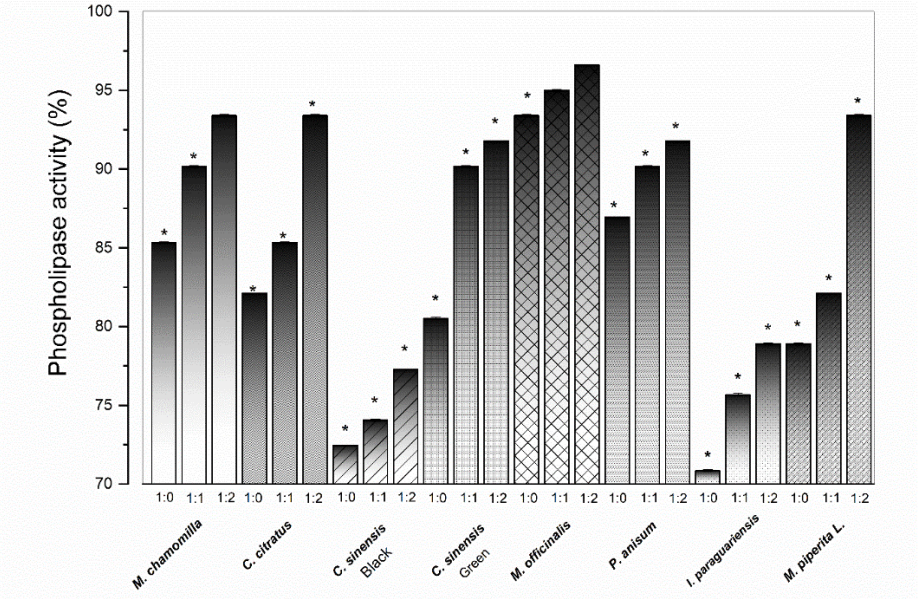


Figure 1. Effect of infusions on the activity of PLA_2s of *Bothrops moojeni* Hoge (10 µg), evaluated after incubation for 30 minutes at 37 °C. The data correspond to means with their respective standard deviations (n=3). The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0, 1:1 and 1:2 tea:PBS (v:v). The mean of the controls containing only venom was considered 100% activity. *Significantly different from the respective positive control, $p<0.05$.

The literature has described the anti-inflammatory action of the plant species *C. sinensis* and *I. paraguariensis*, attributing this effect to the presence of phenolic compounds such as phenolic acids (caffeic, gallic and syringic acids) and, primarily, flavonoids (quercetin, kaempferol, catechin and epigallocatechin gallate) [28, 29]. Plant extracts rich in phenolic compounds are also indicated as PLA_2 inhibitors [30].

The catalytic activity of PLA_2s is dependent on calcium; thus, its inhibition may be associated with the complexation of metal ions (Ca^{2+}) by phenolic chelators (mainly flavonoids) [31]. Flavonoids can also act through hydrogen donation mechanisms to inhibit the action of free radicals, which explains their correlation

with medicinal effects [25, 32]. In addition, phenolic compounds can bind to hydrophobic regions in the structure of these enzymes and/or interact with amino acid residues found in the catalytic sites of phospholipases [33].

Flavonols, such as quercetin and kaempferol, are biomolecules with high potential for the inhibition of PLA_2s, and these are the main phenolic constituents found in yerba mate and black tea [34, 35] whose infusions had the highest PLA_2s inhibition percentages among the tested samples.

In the quantification of flavonoids in yerba mate performed by Bojic [36], the concentrations of quercetin and kaempferol were 2.2 mg g^{-1} and 4.5 mg g^{-1}, respectively. Treatment with quercetin, performed by Cotrim and coauthors [37], led to a reduction of approximately 40% in the PLA_2 activity induced by *Crotalus durissus terrificus* venom. The yerba mate infusion used in the present study was able to inhibit the phospholipase activity by approximately 30%, thus confirming the potential of this herb in the modulation of PLA_2 activity induced by *B. moojeni* venom.

Anti-inflammatory activity evaluated by erythrocyte membrane stability at 54 °C

The significant inhibition of PLA_2 activity conferred anti-inflammatory action to the evaluated herbal infusions. However, the infusions had no inhibitory effect on erythrocyte lysis induced by incubation in a heating bath at 54 °C.

The assay to evaluate the stability of erythrocyte membranes has been considered an indicator of anti-inflammatory potential due to the similarity between erythrocyte and lysosomal membranes, whose contents released by lysis amplify the inflammatory process. However, the anti-inflammatory mechanisms associated with membrane protection have not been described in the literature. For this study, different samples of the species *C. sinensis* (green and black tea) were selected due to the results of the phospholipase and proteolytic activities. The evaluated infusions were able to reduce the production of eicosanoids by inhibiting PLA_2s, resulting in the decreased production of arachidonic acid and consequently of its structural derivatives, although they do not exert a protective action on erythrocyte membranes [34, 35].

In the evaluations performed after incubation at 37 °C, hemolysis percentages between 21 and 24% were observed for the treatments with prednisolone (SAID, steroidal anti-inflammatory drug), between 19 and 24% for the treatment with black tea infusion and between 10 and 13% for the treatment with green tea infusion, compared to the normal mechanical hemolysis rate obtained for the negative control (PBS), which was approximately 4% (Table 1).

Table 1. Evaluation of thermal hemolysis in the presence of anti-inflammatory drugs and infusions of *Camellia sinensis* L. (green and black tea).

Sample	Temperature (°C)	Hemolysis (%)
PBS	**37**	**4.45094***
SAID (25 µg mL^{-1})	37	24.269*
SAID (50 µg mL^{-1})	37	21.9948*
SAID (75 µg mL^{-1})	37	21.0527*
PBS	**37/54**	**22.6446***
NSAID (25 µg mL^{-1})	37/54	20.7927*
NSAID (50 µg mL^{-1})	37/54	22.4172*
NSAID (75 µg mL^{-1})	37/54	22.7087*
***Camellia sinensis* L. (black)**		
1:0	37	19.3023*
1:1	37	24.5289*
1:0	37/54	50.9421*
1:1	37/54	50.2599*
***Camellia sinensis* L. (green)**		
1:0	37	10.1039*
1:1	37	13.3203*
1:0	37/54	55.1332*
1:1	37/54	76. 8356*

The data are the means and standard deviations (n=3). The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0 and 1:1 tea:PBS (v:v), SAID: nimesulide and NSAID: prednisolone. The tests were evaluated in a 2% (v:v) hematocrit solution. The mean of the controls containing only water was considered 100% hemolysis. *Significantly different from the respective positive control, $p<0.05$.

In the hemolysis induction assays at 54 °C, treatment with nimesulide (NSAID, nonsteroidal anti-inflammatory drug) resulted in hemolysis percentages between 20 and 22%, compared to a baseline percentage of approximately 22% for the negative control. Although the treatments with black and green tea

infusions reduced hemolysis induced at 54 °C compared to that induced by the positive control (pure water, 100% hemolysis), the hemolysis percentages were between 50 and 76% (Table 1).

The percentages of hemolysis remained practically constant in the treatments with SAID and NSAID, especially in the concentrations of 50 and 75 μg mL^{-1}, demonstrating that these treatments were not very efficient in controlling hemolysis, even tripling the concentration of the synthetic anti-inflammatory drug from 25 to 75 μg mL^{-1}, at the temperature of induction of hemolysis (54 °C). The results with aliquots of herbal infusions were more expressive in reducing hemolysis in treatments with higher tea:PBS ratio (1:0), which corroborates to the anti-inflammatory and anti-hemolytic potential of bioactive compounds present in these preparations, such as phenolic acids, epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin gallate, gallocatechin, and catechin [3-6].

In this context, it is necessary to highlight that the evaluated samples represent small volumes of teas that may be consumed daily, enhancing their disease prevention and health maintenance effects, which would avoid the adverse actions associated with the use of synthetic anti-inflammatory drugs, especially steroidal drugs.

In some types of fermented teas, such as black tea, monomeric catechins are oxidized or condensed to form theaflavins [38]. Raghava and coauthors [39] highlighted the antihemolytic property of an aqueous extract of *C. sinensis* In addition, a decrease in hemolysis induced by oxidative stress in erythrocytes was reported in the presence of green tea and black tea, with catechins and theaflavins identified as the main compounds responsible for the effect. Reports in the literature point to a possible mechanism that would explain the behavior of the evaluated teas in the membrane stability test.

Several biological effects of theaflavin have been attributed to its antioxidant properties, although the exact mechanism has not been fully elucidated [38, 40]. The theaflavins present in black tea, such as theaflavin-3,3'-digallate, are more effective protease inhibitors than are the catechins present in green tea [41].

C. sinensis is a rich source of compounds that have biological activities. Among them, the phenolic compounds stand out, and moreover the catechins (flavonoids), such as: epigallocatechin gallate, epigallocatechin, epicatechin gallate, epicatechin, gallocatechin gallate, and gallocatechin [5, 28, 35]. The phenolic compounds present in green tea are identified as potent antioxidants, especially the catechins that have the ability to donate hydrogen ions to inhibit the action of free radicals, which justifies their correlation with the medicinal field [5, 28].

Cytotoxic activity on human erythrocytes

In the gel hemolysis assays, the activity of venom, used as a lysis inducer, was inhibited by 50.00% after incubation with yerba mate and 58.33% after incubation with black tea. The green tea, lemon balm and lemongrass infusions had lower inhibitory effects, and the inhibition percentages observed for the highest doses were, respectively, 44.44%, 41.67% and 38.89%. Lower but statistically significant inhibition rates were observed for chamomile (27.78%), anise (25.00%) and peppermint (19.44%) teas. Thus, under the evaluated experimental conditions, the inhibition of hemolysis induced by *B. moojeni* venom was more robust with black tea and yerba mate (Figure 2), as also observed in the phospholipase test.

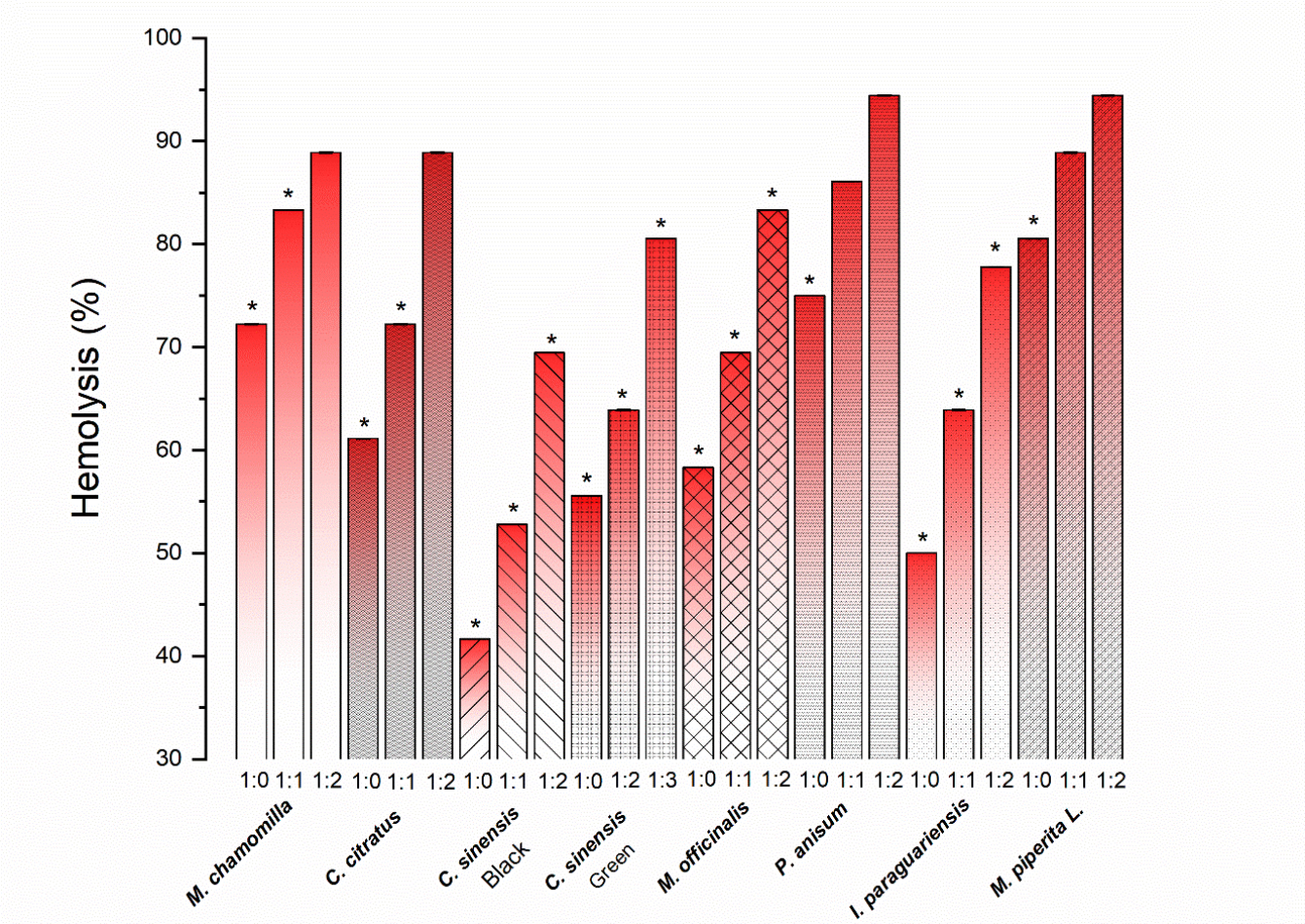


Figure 2. Hemolytic activity (in semisolid medium) induced by *Bothrops moojeni* Hoge venom (10 µg) evaluated after incubation with infusions for 30 minutes at 37 °C. The data are the means and standard deviations (n=3). The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0, 1:1 and 1:2 tea:PBS (v:v). The mean of the controls containing only venom was considered 100% activity. *Significantly different from the respective positive control, $p<0.05$.

Significant amounts of phenolic compounds with hypocholesterolemic, hepatoprotective, diuretic, and antioxidant properties are reported in the dry extract of *I. paraguariensis*, highlighting the derivatives of caffeoyl acid, caffeic acid, quercetin, rutin, and kaempferol [29, 35].

Among the enzyme classes present in the venom used as a hemolysis inducer, metalloproteases with disintegrin domains and regions rich in cysteines, characterized by cytotoxic, hemorrhagic and thrombolytic action, are notable [42, 43]. The inhibitory effect of the teas on hemolytic activity corroborates reports in the literature, in which the rich phenolic composition of plants is associated with protease inhibition [44, 45].

Proteases are associated with the hemorrhagic process, platelet aggregation and coagulation [46]. Naturally occurring nonprotein inhibitors, such as phenolic compounds, undergo hydrophobic interactions with aromatic amino acid residues present in the enzyme structure, in addition to promoting complexation with metal ions [45].

Proteolytic activity on casein

Casein degradation was evaluated using *B. moojeni* venom as a source of proteases, and the inhibitory action of the herbal infusions on the catalysis exerted by proteases was analyzed after incubation with 10 µg of venom for 30 min at 37 °C. The most significant inhibition rates were observed for black tea (40.74%), green tea (31.48%) and yerba mate (25.93%). The other infusions exerted lower but statistically significant inhibition rates relative to the positive control, namely, 14.81% for chamomile, 16.67% for lemongrass, 14.81% for lemon balm and anise, and 12.96% for peppermint (Figure 3).

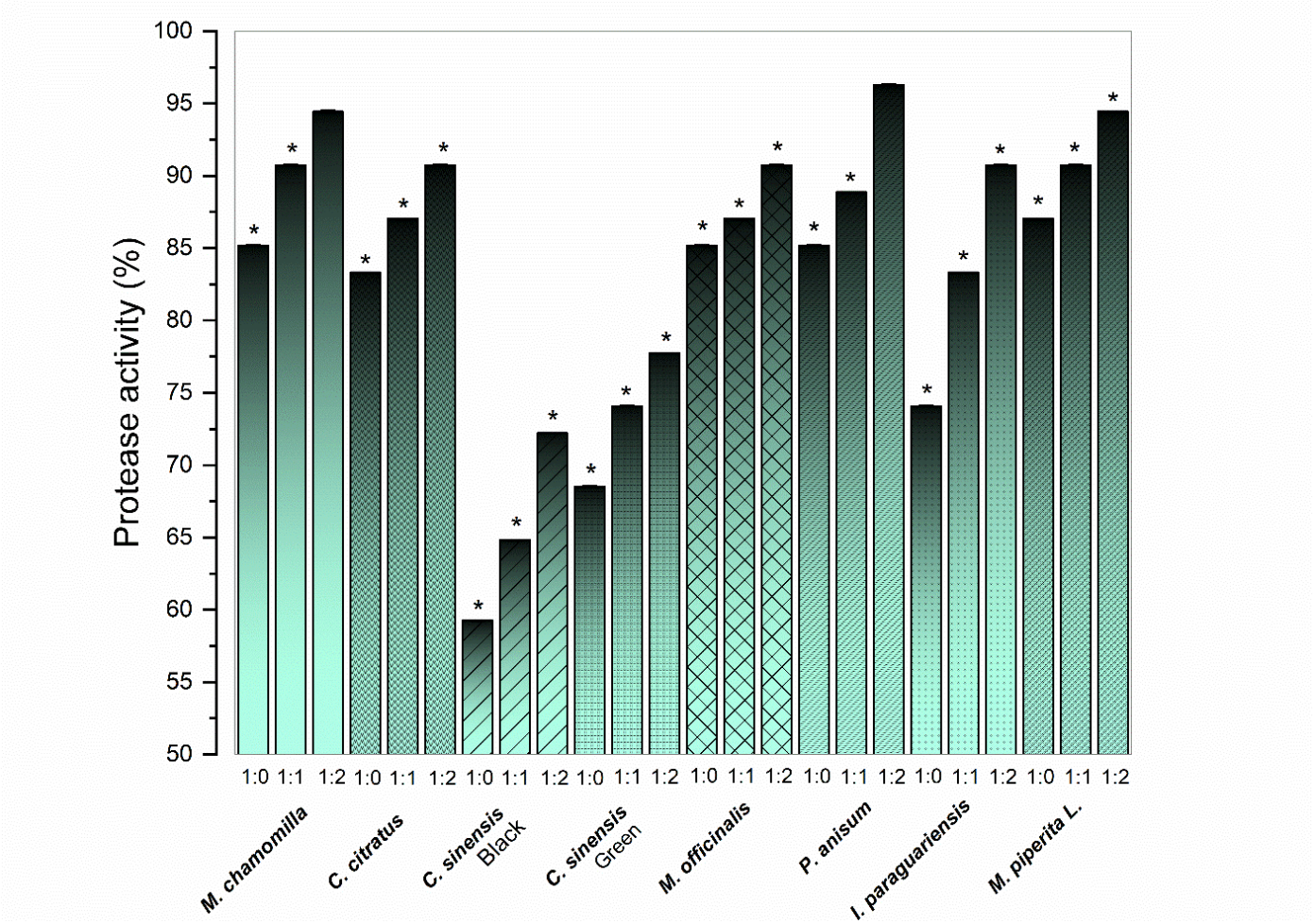


Figure 3. Protease activity of *Bothrops moojeni* Hoge venom (10 µg), evaluated after incubation with the infusions for 30 minutes at 37 °C. The data are the means and standard deviations (n=3). The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0, 1:1 and 1:2 tea:PBS (v:v). The mean of the controls containing only venom was considered 100% activity. *Significantly different from the respective positive control, $p<0.05$.

The relative standard deviations of the cited samples (all <5.00%) indicated agreement among the data. Thus, under the evaluated experimental conditions, the inhibition of the proteases present in *B. moojeni* venom was more robust with black tea, green tea and yerba mate.

The cytotoxic activity inhibition exerted by the teas corroborate the results observed for the proteolytic activity on casein because a large part of the hemolytic action exerted by the venom can be attributed to proteases.

The main molecules of plant origin described in the literature with inhibitory action on proteases, especially those that compose snake venom, are phenolic compounds, such as quercetin, kaempferol, catechins, theaflavins and caffeic and gallic acids [41, 47].

Among the tested samples, infusions of *C. sinensis* (black tea and green tea) were the most efficient in reducing proteolytic activity. Green tea is a rich source of compounds of biological interest, especially phenolic compounds, with catechins found in greater amounts [2, 48].

Furthermore, phenolic compounds present in fermented teas (e.g., black tea), such as tannins, can precipitate proteins and form insoluble complexes with various metal ions (acting as a chelator), which are fundamental cofactors for the activity of phospholipase and proteolytic enzymes, thus reducing the catalytic potential of these classes of enzymes [5, 28, 48].

Fibrinogenolytic activity

The green tea and yerba mate infusions, both at ratios of 1:2 and 1:3 (tea: PBS), were able to decrease, when compared to the control containing venom and fibrinogen, the proteolytic activity induced by *B. moojeni* venom on the α and β chains of fibrinogen; however, yerba mate at a 1:2 ratio was slightly advantageous compared to the other treatments. Thus, this fibrinogenolytic activity inhibition result corroborates the inhibitory action of phenolic compounds present in the teas on the activity of proteases present in the venom.

The fibrinogenases (such as the metalloproteases α-fibrinogenase and β-fibrinogenase) contained in *B. moojeni* venom [11] were inhibited in the presence of the yerba mate and green tea infusions, thus corroborating the results of the proteolytic and hemolytic tests (sections 3.3 and 3.4), which also showed a reduction in the activity of these enzymes. Furthermore, it is believed that the mechanism of fibrinogenase inhibition is similar to that already cited in this study, i.e., cofactor complexation and/or hydrophobic interactions between the bioactive compounds and the catalytic sites [45, 46].

Coagulant activity

The minimum coagulant dose used was 10 µg of *B. moojeni* venom. In the assays with plasma/infusion incubation and the subsequent addition of venom, the coagulation time was prolonged for all samples, with the longest time observed for samples with a 1:0 ratio (Table 2A). The green tea, black tea and lemon balm incubates had coagulation times of approximately twice that for the control group containing only venom.

In the assays with infusion/venom incubation and the subsequent addition of plasma, prolongation of the coagulation time was observed in all samples, with more significant values for the samples prepared with a 1:0 ratio (Table 2B). The treatments with green tea and black tea at a 1:0 ratio showed coagulation times approximately double that of the venom control. In both trials (A and B), black tea stood out, promoting the longest times until the formation of rigid clots, especially in the treatments in which it was preincubated with venom (132.33±4.04 seconds; 1:0 ratio, tea: PBS).

Table 2. Time of citrated plasma coagulation induced by *Bothrops moojeni* Hoge venom, with preincubation of the infusions with plasma and the subsequent addition of venom (A) and preincubation of the infusions with venom and the subsequent addition of plasma (B).

Citrated plasma coagulation time (seconds)		
Control (*Bothrops moojeni* Hoge 10 µg)		60.66±3.05
Infusion + plasma incubation with subsequent venom addition (A)		
Sample	**Ratio Infusion:PBS (v:v)**	**Seconds**
Camellia sinensis L, (green)	1:0	117.00±7.00*
	1:1	79.00±6.24*
Camellia sinensis L. (black)	1:0	127.66±6.50*
	1:1	105.00±10.81*
Ilex paraguariensis A. St.-Hil	1:0	103.00±4.34*
	1:1	98.33±7.50*
Melissa officinalis L.	1:0	121.33±6.11*
	1:1	94.66±4.04*
Infusion + venom incubation with subsequent plasma addition (B)		
Sample	**Ratio Infusion:PBS (v:v)**	**Seconds**
Camellia sinensis L. (green)	1:0	111.33±8.02*
	1:1	101.33±8.14*
Camellia sinensis L. (black)	1:0	132.33±4.04*
	1:1	94.00±8.18*
Ilex paraguariensis A. St.-Hil	1:0	108.33±7.57*
	1:1	106.00±6.08*
Melissa officinalis L.	1:0	94.66±5.03*
	1:1	89.33±7.04*

The data are the means and standard deviations (n=3), $p<0.05$. The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0 and 1:1 tea:PBS (v:v). *Significantly different from the respective positive control, $p<0.05$.

The prospection of phospholipase and protease modulators in plant matrices is of great relevance for the control of hemostatic system disorders due to the action of plant compounds (mainly flavonoids) in the formation of complexes with Ca^{2+}/Zn^{2+} ions, cofactors of enzymes linked to hemostasis, resulting not only in the inhibition of PLA_2s but also in the coagulant action of human proteases that act in the coagulation cascade [31, 43].

Bioactive compounds such as chlorogenic acid, caffeic acid, myricetin, quercetin, kaempferol, rutin, apigenin, luteolin, catechins, tannins, and naringenin present in samples of *M. officinalis* [4, 10], *C. sinensis* [5, 28], and *I. paraguariensis* [29, 35] are reported as agents that delay clotting time, thus demonstrating their pharmacological potential in cases of thrombosis and other disorders associated with the formation of clots throughout the inflammatory process [19, 20].

The aforementioned phenolic compounds most likely play an inhibitory role on the activity of enzymes from the serine proteases and metalloproteases families, as these have a diverse pharmacological profile, which includes actions on the proteins of the coagulation cascade, such as activity similar to the action of thrombin on fibrinogen, factor V and protein C activation, fibrinolysis, plasminogen activation, and induction of platelet aggregation [13, 15, 19].

Compared to the venom control, the incubates, especially those of black tea, showed a significant increase in coagulation time, probably because they act as inhibitors of some enzymes (such as procoagulant proteases), and should be explored regarding their potential application in the treatment of cardiovascular diseases. This opens new possibilities for studies to evaluate the pharmacological potential, efficacy and safety of these herbal infusions.

Activity on blood thrombi

When compared to the control containing only venom (considered 100% thrombus lysis), all controls with pure tea had thrombolytic activity, promoting the dissolution of thrombi at higher rates than that induced by the venom, especially chamomile, with a value 2.7 times higher than that of the venom (273.55% dissolution) (Figure 4).

Conversely, the treatments with lemon balm and yerba mate at the lowest dose and peppermint at the highest dose resulted in thrombotic action, with a reduction in fluid release compared to the negative control (PBS) (Figure 4). This behavior observed for *M. officinalis* and *M. piperita* samples may be associated with thrombotic and bleeding control properties related to phenolic compounds present in herbal infusions [2, 10]. The results observed for *P. anisum* infusions indicate dose-dependence among the samples and their thrombolytic potential in dissolving blood clots, properties associated with the presence of phenolic acids, such as: p-coumaric acid, 5-caffeoylquinic acid, chlorogenic acid, neo-chlorogenic acid and cryptochlorogenic acid [3-8].

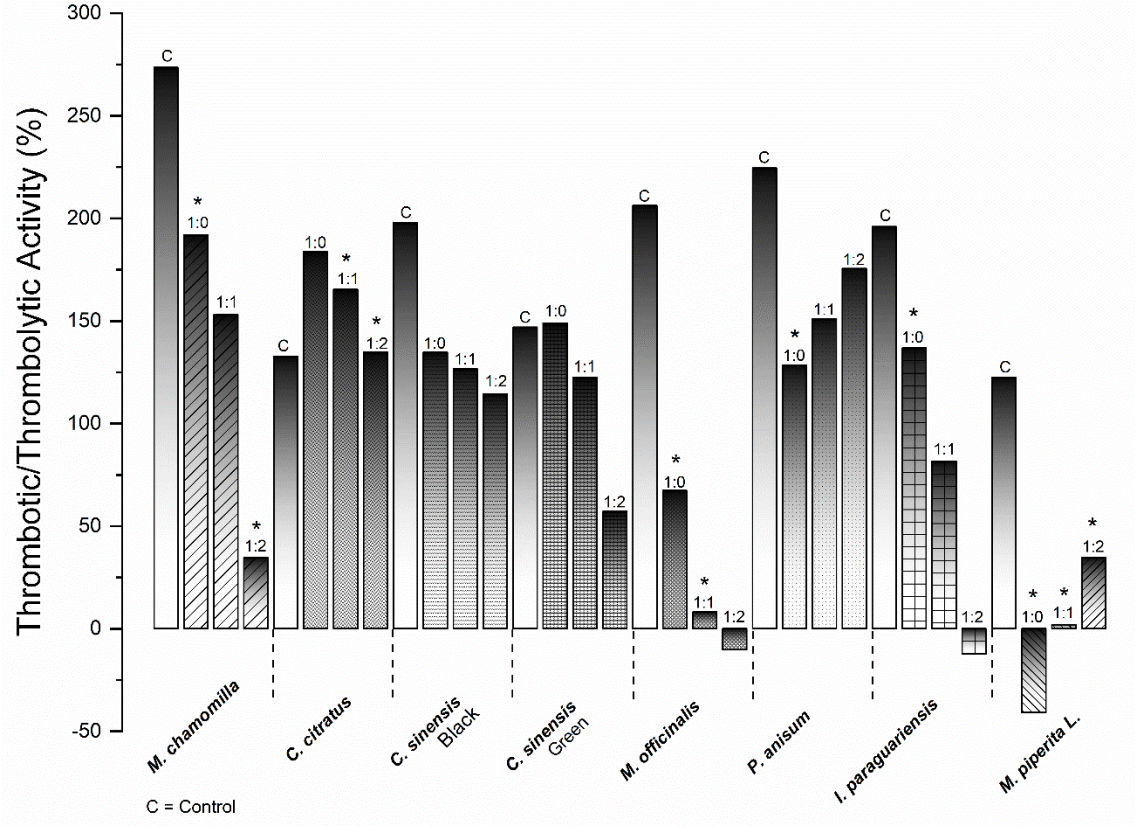


Figure 4. Effect of herbal infusions on the dissolution of thrombi formed by *Bothrops moojeni* Hoge venom (30 µg), evaluated after incubation for 30 minutes at 37 °C. The data are the means and standard deviations (n=3). The ratios correspond to the infusion volume relative to the total reaction volume, with 1:0, 1:1 and 1:2 tea:PBS (v:v). The mean of the controls containing only venom (positive control) was considered 100% activity. The mean fluid volume released by the thrombi treated only with PBS (negative control) was deducted from the other treatments. C = controls treated only with the infusions, without venom. *Significantly different from the respective positive control, $p<0.05$.

The lysis of blood thrombi is associated with proteases with hemorrhagic, fibrin(ogen)olytic and cytotoxicity actions, whose structures have, in most cases, disintegrin- and cysteine-rich domains [49]. Although the same enzyme classes in venom are associated with thrombolysis and hemolysis, the performance pattern of the infusions in the hemolysis assay was different from the pattern observed in the thrombolytic assay, although both results point to the presence of protease inhibitors in the evaluated infusions. This result can be explained by the presence of compounds in the infusions that affect the integrity of thrombi, such as antiplatelet agents, fibrinolytic agents and plasminogen activators [50].

In addition, the diversity of molecules present in both the infusions and venom must be considered; these molecules act on different substrates, being only free erythrocytes in the hemolysis assay and a complex network of molecules (lipids, carbohydrates and proteins) and cells (erythrocytes and leukocytes) in the test evaluating effects on thrombi (prepared with whole blood from non-fasted volunteers).

The bioactive substances contained in plant extracts can alter the shape of the coordination sites of cofactors present in the protease structures, thus affecting cofactor binding and, as a result, altering the catalytic properties of the enzymes. Secondary metabolites of plant extracts are reported as neutralizing agents of the hemorrhagic effect induced by *Bothrops asper* Garman venom; in addition, flavonoid-rich extracts are capable of chelating ions such as Zn^{2+} that are essential for the enzymatic activity of metalloproteases [44, 51].

Thus, the significant reduction in both proteolytic activity and hemolytic activity in the presence of black tea may be associated with the action of these biomolecules in protease modulation. Although there are several studies proving the health benefits of phenolic compounds through ingestion, little is known about the activity of their metabolites in biological systems. Furthermore, evaluations on the effectiveness of bioactive compounds are usually performed *in vitro*, investigating the “parent compound”, but not the synergistic action with circulating metabolites [2, 3, 10]. In this way, the need for further studies is pointed out to understand the action of these compounds, as well as the benefits of the products of their metabolism in the human body.

CONCLUSION

The herbal infusions showed potential for nutraceutical use and may have an adjuvant action in the treatment of diseases with inflammatory origin or progression, with effects likely associated with the content of phenolic compounds, especially flavonoids, present in the teas.

Phospholipase activity was inhibited by more than 25% in the presence of black tea and yerba mate tea, with the flavonoids quercetin and kaempferol identified as possibly responsible for the modulation of PLA_2s, as they are present in significant amounts in these infusions, as previously described in the literature. The hemolytic activity was significantly reduced in the treatments with infusions of *C. sinensis* (green and black tea) and *I. paraguariensis* (yerba mate); thus, the reduction in hemolysis may be associated with the hydrophobic interactions between flavonoids and the aromatic residues of amino acids present in the structure of the enzymes, in addition to possible complexation with their cofactors. Protease inhibition was more effective in treatments using black tea and green tea, with the phenolic compounds quercetin, kaempferol, catechins and theaflavins indicated as the likely modulators. Black tea stood out among the others by delaying plasma coagulation longer, especially after pretreatment with venom.

Thus, it is possible to conclude that routine consumption of herbal infusions, such as black tea, green tea and yerba mate, could result in benefits to human health, due to both the high amount of bioactive antioxidant compounds that exert effects in the prevention of diseases the ability of those compounds to modulate the activity of enzymes linked to the hemostatic system, with possible benefits as complementary treatments for inflammatory and cardiovascular diseases.

Acknowledgments: The authors are grateful for financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Fundação de Amparo à Pesquisa de Minas Gerais (CNPq/CAPES/FAPEMIG), Brazil.
Conflicts of Interest: The authors declare that they have no conflicts of interest.

REFERENCES

1. Zhao L, Sun Q-Y, Ge Z-J. Potential role of tea extract in oocyte development. Food Funct. 2021 Nov;12(21):10311-23.
2. Pinto MD. Tea: A new perspective on health benefits. Food Res. Int. 2013 Oct;53(2):558-67.
3. Shi J, Yang G, You Q, Sun S, Chen R, Lin Z, et al. Updates on the chemistry, processing characteristics, and utilization of tea flavonoids in last two decades (2001-2021). Crit. Rev. Food Sc.i Nutr. 2021 Dec; 13:1-28.
4. Yin DD, Yuan RY, Wu Q, Li SS, Shao S, Xu YJ, et al. Assessment of flavonoids and volatile compounds in tea infusions of water lily flowers and their antioxidant activities. Food Chem. 2015 Nov;187:20-8.

5. Samanta S. Potential bioactive components and health promotional benefits of tea (*Camellia sinensis*). J. Am. Nutr. Assoc. 2022 Jan;41(1):65-93.
6. Wu M, Cai J, Fang Z, Li S, Huang Z, Tang Z, et al. The Composition and anti-aging activities of polyphenol extract from *Phyllanthus emblica* L. fruit. Nutrients. 2022 Feb 18;14(4):857.
7. Morais SM, Cavalcanti ESB, Costa SMO, Aguiar LA. Antioxidant action of teas and seasonings more consumed in Brazil. Braz. J. Pharmacog. 2009;19(1):315-20.
8. Burkard M, Leischner C, Lauer UM, Busch C, Venturelli S, Frank J. Dietary flavonoids and modulation of natural killer cells: implications in malignant and viral diseases. J. Nutr. Biochem. 2017 Aug;46:1-12.
9. Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, et al. Role of phenolic compounds in human disease: current knowledge and future prospects. Molecules. 2021 Dec 30;27(1):233.
10. Correa VG, Tureck C, Locateli G, Peralta RM, Koehnlein EA. Estimate of consumption of phenolic compounds by Brazilian population. Braz. J. Nutr. 2015;28:185-96.
11. Zhang Y. Why do we study animal toxins? Dongwuxue Yanjiu. 2015 Jul 18;36(4):183-222.
12. Gutiérrez J, Avila C, Rojas E, Cerdas L. An alternative *in vitro* method for testing the potency of the polyvalent antivenom produced in Costa Rica. Toxicon. 1988;26:411–3.
13. Nkeh-Chungag BN, Oyedeji OO, Oyedeji AO, Ndebia EJ. Anti-inflammatory and membrane-stabilizing properties of two semisynthetic derivatives of oleanolic acid. Inflammation. 2015 Feb;38(1):61-9.
14. Anil UT, Ajay KS. Further studies on membrane stabilizing, anti-inflammatory and FCA induced arthritic activity of various fractions of bark of *Machilus macrantha* in rats. Rev. Bras. Farmacogn. 2011 Nov; 21(6):1052-64.
15. WIlliams LA, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, et al. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med. J. 2008 Sep;57(4):327-31.
16. Preté PSC, Domingues CC, Meirelles NC, Malheiros SVP, Goñi FM, De Paula E, et al. Multiple stages of detergent-erythrocyte membrane interaction—A spin label study. Biochim. Biophys. Acta. 2011 Jan;1808(1):164-70.
17. Wang WJ, Shih CH, Huang TF. A novel P-I class metalloproteinase with broad substrate-cleaving activity, agkislysin, from *Agkistrodon acutus* venom. Biochem Biophys Res Commun. 2004 Nov 5;324(1):224-30.
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature.1970 Aug 15;227(5259):680-5.
19. Rodrigues VM, Soares AM, Guerra-Sá R, Rodrigues V, Fontes MR, Giglio JR. Structural and functional characterization of neuwiedase, a nonhemorrhagic fibrin(ogen)olytic metalloprotease from *Bothrops neuwiedi* snake venom. Arch. Biochem. Biophys. 2000 Sep 15;381(2):213-24.
20. Selistre HS, Queiroz LS, Cunha OAB, Souza GEP, Giglio JR. Isolation and characterization of hemorrhagic, myonecrotic and edema-inducing toxins from *Bothrops insularis* (jararaca ilhoa) snake venom. Toxicon. 1990;28(3):261-73.
21. Cintra ACO, Toni LGBD, Sartim MA, Franco JJ, Caetano RC, Murakami MT, et al. Batroxase, a new metalloproteinase from *B. atrox* snake venom with strong fibrinolytic activity. Toxicon. 2012 Jul;60(1):70-82.
22. R Core Team. R: A Language and Environment for Statistical Computing. Viena: R Foundation for Statistical Computing. 2012.
23. Guimarães CL, Moreira-Dill LS, Fernandes RS, Costa TR, Hage-Melim LI, Marcussi S, et al. Biodiversity as a source of bioactive compounds against snakebites. Curr. Med. Chem. 2014;21(25):2952-79.
24. Carvalho BMA, Santos JDL, Xavier BM, Almeida JR, Resende LM, Martins W, et al. Snake venom PLA_2s inhibitors isolated from Brazilian plants: synthetic and natural molecules. Biomed. Res. Int. 2013;2013:153045.
25. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: A review. Molecules 2022 May 2;27(9):2901.
26. Zaragozá C, Álvarez-Mon MA, Zaragozá F, Villaescusa L. Flavonoids: antiplatelet effect as inhibitors of COX-1. Molecules. 2022 Feb 8;27(3):1146.
27. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutr. J. 2016 Dec 1;15(1):99.
28. Bedrood ZMR, Hosseinzadeh H. Toxicological effects of *Camellia sinensis* (green tea): a review. Phytother. Res. 2018 Jul;32(7):1163-80.
29. Piovezan-Borges AC, Valério-Júnior C, Gonçalves IL, Mielniczki-Pereira AA, Valduga AT. Antioxidant potential of yerba mate (*Ilex paraguariensis*) extracts in *Saccharomyces cerevisae* deficient in oxidant defense genes. Braz. J. Biol. 2016 Jun;76(2):539-44.
30. Braga MA, de Abreu TS, Trento MVC, Machado GHA, Pereira LLS, Simão AA, et al. Prospection of enzyme modulators in aqueous and ethanolic extracts of *Lippia sidoides* leaves: genotoxicity, digestion, inflammation, and hemostasis. Chem. Biodivers. 2019 Mar;16(3):e1800558.
31. Moura VM, Silva WC, Raposo JD, Freitas-de-Sousa LA, Dos-Santos MC, Oliveira RB, et al. The inhibitory potential of the condensed-tannin-rich fraction of *Plathymenia reticulata* Benth. (Fabaceae) against *Bothrops atrox* envenomation. J. Ethnopharmacol. 2016 May;183:136-42.
32. Sun ZG, Li ZN, Zhang JM, Hou XY, Yeh SM, Ming X. Recent developments of flavonoids with various activities. Curr. Top. Med. Chem. 2022 Mar;22(4):305-29.

33. Teixeira ML, Marcussi S, DE CSR, Danubia A, Magalhães ML, Nelson DL, et al. Essential oil from *Lippia origanoides* (Verbenaceae): haemostasis and enzymes activity alterations. Med. Chem. 2019;15(2):207-14.
34. Coutinho MAS, Muzitano MF, Costa SS. Flavonoids: potential therapeutic agents for the inflammatory process. Rev. Virt. Quim. 2009;1:241-56.
35. Peluso I, Serafini M. Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. Br. J. Pharmacol. 2017 Jun; 174(11):1195-208.
36. Bojic M, Haas VS, Saric D, Males Z. Determination of flavonoids, phenolic acids, and xanthines in mate tea (*Ilex paraguariensis*). J. Anal. Methods Chem. 2013;2013:658596.
37. Cotrim CA, Oliveira SCB, Filho EBD, Fonseca FV, Baldissera JR L, Antunes E, et al. Quercetin as an inhibitor of snake venom secretory phospholipase A_2. Chem. Biol. Interact. 2011 Jan;189(1-2):9-16.
38. Bancirova M. Comparison of the antioxidant capacity and the antimicrobial activity of black and green tea. Food Res. Int. 2010 Jun; 43(5):1379-82.
39. Raghava KP, Hakira A, Kiranmai GS, Reddy YH, Kartheek C, Babu AMSS. Evaluation of anti-hemolytic activity of green tea aqueous extracts by *in vitro* method. Int. J. Res. Pharmacol. Pharmacother. 2017;6(2):191-8.
40. Shan Z, Nisar MF, Li M, Zhang C, Wan CC. Theaflavin chemistry and its health benefits. Oxid. Med. Cell Longev. 2021 Nov;2021:6256618.
41. Chen CN, Lin CP, Huang KK, Chen WC, Hsieh HP, Liang PH, et al. Inhibition of SARS-CoV 3C-like protease activity by theaflavin-3, 3'-digallate (TF3). Evid. Based Complement. Alternat. Med. 2005 Jun;2(2):209-15.
42. Oliveira CH, Simão AA, Trento MV, César PH, Marcussi S. Inhibition of proteases and phospholipases A_2 from *Bothrops atrox* and *Crotalus durissus terrificus* snake venoms by ascorbic acid, vitamin E, and B-complex vitamins. An. Acad. Bras. Cienc. 2016;88(3 Suppl):2005-16.
43. Cate HT, Hackeng TM, Frutos PG. Coagulation factor and protease pathways in thrombosis and cardiovascular disease. J. Thromb. Haemost. 2017 Jun;117(7):1265-71.
44. Patiño AC, Benjumea DM, Pereañez JA. Inhibition of venom serine proteinase and metalloproteinase activities by *Renealmia alpinia* (Zingiberaceae) extracts: Comparison of wild and *in vitro* propagated plants. J. Ethnopharmacol. 2013 Sep;149(2):590-6.
45. Saavedra SL, Avila L, Giudicessi SL, Albericio F, Camperi SA, Cascone O, et al. Natural snake venom inhibitors and their pharmaceutical uses: challenges and possibilities. Curr. Pharm. Des. 2018;24(16):1737-47.
46. Queiroz MR, Sousa BB, Pereira DFC, Mamede CCN, Matias MS, Morais NCG, et al. The role of platelets in hemostasis and the effects of snake venom toxins on platelet function. Toxicon. 2017 Jul;133:33-47.
47. Xue G, Gong L, Yuan C, Xu M, Wang X, Jiang L, et al. Structural mechanism of flavonoids in inhibiting serine proteases. Food Funct. 2017Jul;8(7):2437-43.
48. Tyagi T, Garlapati PK, Yadav P, Naika M, Mallya A, Kandangath Raghavan A. Development of nano-encapsulated green tea catechins: Studies on optimization, characterization, release dynamics, and *in-vitro* toxicity. J. Food Biochem. 2021 Nov;45(11):e13951.
49. Baraldi PT, Magro AJ, Matioli FF, Marcussi S, Lemke N, Calderon LA, et al. A novel synthetic quinolinone inhibitor presents proteolytic and hemorrhagic inhibitory activities against snake venom metalloproteases. Biochimie. 2016 Feb;121:179-88.
50. Al-Horani RA, Afosah DK. Recent advances in the discovery and development of factor XI/XIa inhibitors. Med. Res. Rev. 2018 Sep;38(6):1974-2023.
51. Oliveira CHM, Simão AA, Marcussi S. Inhibitory effects of ascorbic acid, vitamin E, and vitamin B-complex on the biological activities induced by *Bothrops* venom. Pharm. Biol. 2016;54(5):845-52.