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INTERFACES CIENTÍFICAS

MODULATING ACTION OF AROMATIC PLANTS AND PROBIOTICS ON ENZYME ACTIVITY

AÇÃO MODULADORA DE PLANTAS AROMÁTICAS E PROBIÓTICOS SOBRE A ATIVIDADE DE ENZIMAS

ACCIÓN MODULADORA DE PLANTAS AROMÁTICAS Y PROBIÓTICOS SOBRE LA ACTIVIDAD ENZIMÁTICA

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ABSTRACT

The present study aimed to evaluate the effects of extracts from aromatic plants and probiotics on the enzymatic activities induced by *Bothrops moojeni* venom, as well as to assess their potential as anti-inflammatory agents, using *in vitro* tests. The venom's phospholipase and hemolysis activities were found to be inhibited by incubation with pure plant extracts or probiotics at different pH levels. Additionally, all plant extracts with and without probiotics displayed an inhibitory effect on heat-induced hemolysis and venom-induced thrombus lysis. The greatest inhibitions were observed for extracts of *Laurus nobilis* and *Ocimum basilicum*. Furthermore, the extracts of *L. nobilis, O. basilicum* and *Origanum vulgare* with and without probiotics were found to exert an inhibitory action on coagulant enzymes present in the venom, prolonging the clotting time. Interestingly, the *Petroselium crispum* extract in the presence of the probiotic appeared to potentiate the coagulant effect of the venom's enzymes, as observed by a reduction in the clotting time. The findings demonstrate the potential of these plants to modulate the action of enzymes that play a crucial role in the regulation of hemostasis and the inflammatory response, since the enzymes used as laboratory tools show high structural and functional homology with human enzymes.

KEYWORDS

Hemostasis; Aromatic Plants; Probiotic Bacteria. Enzyme Modulators.

RESUMO

O presente estudo teve como objetivo avaliar os efeitos de extratos de plantas aromáticas e probióticos nas atividades enzimáticas induzidas pelo veneno de *Bothrops moojeni*, bem como avaliar seu potencial como agente antiinflamatório, por meio de testes in vitro. Descobriu-se que as atividades de fosfolipase e hemólise do veneno são inibidas pela incubação com extratos puros de plantas ou probióticos em diferentes níveis de pH. Além disso, todos os extratos vegetais com e sem probióticos apresentaram efeito inibitório na hemólise induzida pelo calor e na lise do trombo induzida pelo veneno. As maiores inibições foram observadas para os extratos de *Laurus nobilis* e *Ocimum basilicum*. Além disso, constatou-se que os extratos de *L. nobilis, O. basilicum* e *Origanum vulgare* com e sem probióticos exercem ação inibitória sobre enzimas coagulantes presentes no veneno, prolongando o tempo de coagulação. Curiosamente, o extrato de *Petroselium crispum* na presença do probiótico pareceu potencializar o efeito coagulante das enzimas do veneno, conforme observado pela redução do tempo de coagulação. Os resultados demonstram o potencial dessas plantas em modular a ação de enzimas que desempenham papel crucial na regulação da hemostasia e na resposta inflamatória, uma vez que as enzimas utilizadas como ferramentas laboratoriais apresentam alta homologia estrutural e funcional com enzimas humanas.

PALAVRAS-CHAVE

Hemostasia. Plantas Aromáticas. Bactérias Probióticas. Moduladores Enzimáticos.

RESUMEN

El presente estudio tuvo como objetivo evaluar los efectos de extractos de plantas aromáticas y probióticos sobre las actividades enzimáticas inducidas por el veneno de *Bothrops moojeni*, así como evaluar su potencial como agente antiinflamatorio, mediante pruebas in vitro. Se descubrió que las actividades de fosfolipasa y hemólisis del veneno se inhibían mediante la incubación con extractos puros de plantas o probióticos a diferentes niveles de pH. Además, todos los extractos de plantas con y sin probióticos mostraron un efecto inhibidor sobre la hemólisis inducida por el calor y la lisis de trombos inducida por veneno. Las mayores inhibiciones se observaron para los extractos de *Laurus nobilis* y *Ocimum basilicum*. Además, se encontró que los extractos de *L. nobilis, O. basilicum* y *Origanum vulgare* con y sin probióticos ejercen una acción inhibidora sobre las enzimas coagulantes presentes en el veneno, prolongando el tiempo de coagulación. Curiosamente, el extracto de Petroselium crispum en presencia del probiótico pareció mejorar el efecto coagulante de las enzimas del veneno, como se observó por la reducción del tiempo de coagulación. Los resultados demuestran el

potencial de estas plantas para modular la acción de enzimas que juegan un papel crucial en la regulación de la hemostasia y la respuesta inflamatoria, ya que las enzimas utilizadas como herramientas de laboratorio muestran una alta homología estructural y funcional con las enzimas humanas.

PALABRAS CLAVE

Hemostasia. Plantas aromáticas. Bacterias probióticas. Moduladores enzimáticos

1 INTRODUCTION

The use of medicinal plants for the treatment of various diseases, including hemostatic disorders, has a long history. Hemostatic disorders such as hemorrhages and thrombosis can lead to serious complications. Phenolic compounds found in plants are know to play a signigicant role in their diverse actions on human health. Aromatic plants such as *Laurus nobilis*, *Ocimum basilicum*, *Origanum vulgare* and *Petroselium crispum* have been shown to possess anti-inflammatory, antioxidant, hypoglycemic and antimicrobial properties (LEE *et al*., 2019, TAVALLALI *et al*., 2019, LIBERAL *et al*., 2020, OVIDI *et al*., 2021, YU *et al*., 2021a).

Probiotics, which are live microorganisms that confer health benefits when consumed in adequate amounts, have been shown to play a crucial role in maintaining the balance of the gut microbiota. Probiotic bacteria are commonly used to alleviate intestinal disorders, by exerting a variety of beneficial effects on the host, such as controlling the growth of pathogenic microorganisms, improving gut barrier function, and modulating the immune system. Among the most commonly used probiotic strains are those belonging to the genera L*actobacillus* and *Bifidobacterium.* These microorganisms commonly used in the food industry to manufacture various products such as fermented dairy, probiotic beverages, and dietary supplements (JEZEWSKA-FRACKOWIAK *et al*., 2018).

Snake venom is a complex mixture of biologically active compounds, including enzymes such as phospholipases A2 (PLA2s), metalloproteases and serine proteases, which have been shown to play a critical role in the venom's toxicity by promoting coagulation or bleeding and participating in the inflammatory response. These enzymes share structural and functional similarities with their human counterparts. In this sense, the present work aimed to evaluate the modulating action of aqueous extracts obtained from leaves of *L. nobilis, O. basilicum, O. vulgare* and *P. crispum* in the presence and absence of probiotics, on activities induced by PLAs and proteases, using *in vitro* tests, and direct their potential for use as future preventive agents, after a broad scientific characterization, in the context of inflammatory diseases and hemostatic disorders.

2 MATERIALS AND METHODS

2.1 OBTAINING AND PREPARING SAMPLES

The aromatic plants of the species, *Laurus nobilis, Ocimum basilicum, Origanum vulgare* and *Petroselium crispum* were commercially purchased at a market in the city of Lavras, MG, Brazil. The dried leaves, crushed and packaged by a food industry (according to MAPA regulations – Brazilian Ministry of Agriculture, Livestock and Supply), were used with the aim of simulating the popular consumption of these spice plants. The dried leaves were crushed, sieved, weighed 4 g and dissolved in 40 mL of PBS (phosphate buffered saline solution, pH 7.4, 2 mM NaH2PO4, 3 mM Na2HPO4, 154 mM NaCl) and remained under agitation for 12 h. Then, they were taken to the ultrasound machine for 1h10min. After this step, the solution was centrifiged for 10 min. at 3.5 g and the supernatant was collected and frozen until the moment of use in the assays.

The probiotic bactéria (*Lactobacillus acidophilus, L. rhamnosus, L. paracasei* and *Bifidobacterium lactis*) in powder form, were purchased under the trade name Probiatop. Each 1 g was diluted in 10 mL of PBS and then quantified using the McFarland scale method (ZAMORA; PÉREZ-GRACIA, 2012). Subsequently, a screening was performed with different values of colony-forming units (CFU), to identify ideal amounts of probiotic bacteria to be used in the tests, so that they would not saturate the reaction environment. Then, the bacteria were diluted in PBS pH 7.4 and 8.0 for the phospholipases A (PLAs) activity and PBS pH 7.4 for the other activities.

2.2 SNAKE VENOMS

The crystallized venom of *Bothrops moojeni* (registered in SISGEN under the number ADF95EA) was purchased commercially from the serpentarium Bioagents (Batatais-SP). At room temperature, the crude venom was weighed 10 mg, dissolved in 1.0 mL of PBS and stored at -20 ºC.

All activities [except anti-inflammatory (without the use of venom)] were also performed with plants extracts and probiotics alone. Extracts and probiotic bacteria were pre-incubated with enzyme source (venom) in different proportions for 30 minutes at 37 ºC.

2.3 OBTAINING HUMAN BLOOD

The blood was obtained from healthy volunteers and collected in tubes containing heparin (anti--inflammatory and hemolysis activities), citrate (coagulant activity), and without anticoagulant (thrombolytic activity). All tests using human blood or its components were carried out with the previous authorization of the Ethics Committee on Human Research (COEP) of the Federal University of Lavras, under the registration number: CAAE: 57151322.4.0000.5148.

2.4 IDENTIFICATION OF PHENOLIC COMPOUNDS BY HPLC

Phenolic compounds were identified and quantified for each extract. The phenolic standards used were obtained from Sigma-Aldrich (St. Louis, MO, EUA). High performance liquid chromatography (HPLC) was performed using a Shimadzu UHPLC chromatograph (Shimadzu Corporation, Kyoto, Japão). Equipped with two LC-20AT hogh pressure pumps, an SPD-M20A Uvvis detector, a CTO-20AC oven, a CBM- Interface 20 A, and an auto-injector with a SIL-20A autosampler. Separations were performed using a VP-ODS-C18 (250 mm x 4.6 mm) Shim-pack column, attached to a Shim-pack pre--column (10 mm × 4.6 mm) (Shimadzu, Japão).

The addition of patterns to the extracts was also used as an identification parameter. Stock standard solutions were prepared in methanol (HPLC grade; Sigma-Aldrich, EUA). Extracts and standards were filtered through a 0.45 µm nylon membrane (EMD Millipore, USA) and injected directly into the chromatographic system, in three replications. The phenolic compounds in the extracts were identified by comparing the retention times of the standards. Quantification was performed by constructing analytical curves obtained through linear regression using Origin 6.1 computer software (OriginLab, Northampton, MA, USA) and considering the coefficient of determination (R2) equal to 0.99.

2.5 ANTI-INFLAMMATORY ACTIVITY

To carry out the anti-inflammatory activity adapted from Williams *et al*. (2008), Tatiya and Saluja (2011) and Nkeh-Chungag *et al*. (2015), the blood of the volunteers was collected in tubes containing heparin. The blood was then centrifuged at 1500 *xg* for 5 min., and the plasma removed. The packed red blood cells were used to prepare a 2 % (v/v, mL/mL) suspension in PBS, pH 7.4. The extracts at doses of 1.75 and 3.5 mg and the probiotic bacteria $9.6x10^5$ CFU were previously prepared in volume of 200 µL and then 800 µL of the red blood cell suspension was added. Controls were performed with steroidal anti-inflammatory drug prednisolone [126 µg and 180 µg (anti-inflammatory action as a comparison parameter)], PBS (corresponding to mechanical hemolysis), and water (corresponding to total hemolysis – 100 % hemolysis).

The suspensions containing the samples were incubated at 37 ºC for 30 min. After incubation, the tubes containing the incubated remained in a thermostatic bath at 54 ºC for 20 min., followed by centrifugation at 1200 *xg* for 10 min. Then, the absorptions were read in a spectrophotometer at 540 nm. The protective capacity of the extracts against temperature-induced hemolysis was evaluated. The anti--inflammatory potential of plant extracts previously incubated with probiotic bacteria was also evaluated. The calculation of the percentage of inhibition of hemolysis was done using the following formula:

$$\%\, Hemolysis = 100 - \{\, 1 - \frac{(AHS - AUS)}{(\ C + \ - AUS)} \quad x\ 100 \,\}$$

AHS - absorbance of the heated sample
AUS - absorbance of the unheated sample
C+ - positive control absorbance

To infer possible interferences, present in the extracts, capable of performing absorption at the wavelength used for the evaluation of the tests, a scan of each extract was performed before the thermal hemolysis tests.

2.6 PHOSPHOLIPASE AND HEMOLYSIS ACTIVITY

The phospholipase and hemolysis activities were evaluated in a solid medium, as described by Gutiérrez *et al*. (1988). The gel for the evaluation of the phospholipase activity was prepared with 0.01 mol L^{-1} $CaCl_2$, egg yolk lecithin's (phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine) 1:3 v/v, PBS, 1 % bacteriological agar, and 0.005 % sodium azide. The medium was poured into Petri dishes at 45-50 °C. After gel solidification, the treatments in a final volume of 30 µL diluted in PBS, were applied in 0.5 cm diameter holes made in gel, and the dishes were maintained in a cell culture chamber for 12 hours at 37 °C.

The gel for hemolysis activity was prepared by replacing egg yolk lecithin's for human erythrocyte concentrate calculated to a hematocrit of 1 %. Therefore, the newly collected blood was centrifuged at 900 *xg* for 5 min. to obtain the erythrocytes. The erythrocytes were suspended in 5 mmol L^{-1} of PBS (pH 7.4) and centrifuged under the same conditions, repeating this washing step twice, and then diluted to 1%. The results of both tests were evaluated by measuring the diameter of the translucent halos formed in the gels around the holes where the samples were applied. Inhibition of PLAs and hemolysis activities were evaluated on *B. moojeni* venom 10 and 20 µg, respectively. The venom was previously incubated with extracts of *L. nobilis, O. basilicum, O. vulgare, P. crispum* at doses (0.1, 0.4, 0.7, 1.8, 2.5 mg) and with probiotic bacteria ($2.6x10^5$ CFU) for 30 min. at 37 °C. Dexamethasone and Prednisolone were used at doses of 2.5 µg, 5 µg and 10 µg to compare with the effects of the extracts.

2.7 COAGULANT ACTIVITY

The evaluation of clotting time was performed according to Rodrigues *et al*. (2000). The pure extracts at doses of 0.1, 0.4, 0.7, 1.8, 2.5 mg, and associated with probiotic bacteria $2.6x10^5$ CFU, were previously incubated with *B. moojeni* venom (10 µg) for 30 min. at 37 ºC. Controls containing extracts with and without probiotics, and pure venom alone were also performed. Tubes containing human citrated plasma were kept in a 37 °C bath. The plasma was obtained after centrifugation of freshly collected blood in tubes containing citrate. The previously incubated samples were added to the plasma (200 µL), and then the time until the formation of a rigid clot was measured. The minimum coagulant dose was previously defined, which was the lowest amount of venom capable of inducing plasma coagulation in a range between 50 and 180 seconds.

2.8 THROMBOLYTIC ACTIVITY

The thrombolytic activity was assessed on human blood clots formed *in vitro*, according to the methodology described by Cintra *et al*. (2012). The clots, 100 µL of freshly collected blood distributed in microplate wells, were incubated for 24 h at 37 ºC with the samples. The controls were prepared in a volume of 30 µL, containing *B. moojeni* venom (30 µg) alone, probiotic bacteria $2.6x10^5$ CFU alone, and plant extracts alone, all diluted in PBS. The samples were prepared with venom previously incubated (30 min at 37 ºC) with the pure extracts at doses 0.4, 0.7, 1.8, 2.5 mg, and with these added probiotics.

The activities were calculated by measuring the volume of fluid released by each thrombus, using automatic pipettes with variable calibration. The data obtained in volumes were converted in percentages. The final volume applied to each well, corresponding to blood + sample, 130 µL, was considered as 100 % activity (100% thrombus dissolution). The mean value of controls containing only PBS were subtracted from the means of incubated samples.

2.9 STATISTICAL ANALYSIS

The results were presented as the mean of triplicates ± standard deviation. The data were evaluated by analysis of variance, and the means were compared using the Scott Knott test ($p<0,05$) (R CORE TEAM, 2012).

3 RESULTS

3.1 IDENTIFICATION OF PHENOLIC COMPOUNDS BY HPLC

In the present work, significant amounts of phenolic compounds were found in the extracts of *Laurus nobilis, Ocimum basilicum, Origanum vulgare,* and *Petroselium crispum* (Table 1). Caffeic acid was found in the extracts of *L. nobilis* and *P. crispum* and, in greater concentration, in the extract of *O. vulgare*.

Caffeic acid can exert ant-inflammatory effects by inhibiting cyclooxygenase 2 and one of its products, prostaglandin 2 (ZIELINSKA *et al*., 2021). The catechin identified in the extracts of *L. nobilis* and *O. basilicum* was previously described with microbicidal action on pathogenic bacteria favoring the multiplication of beneficial bacteria (XUE *et al*., 2016).

Table 1 – Identification of phenolic compounds by HPLC in aromatic plants

Phenolic compounds (mg $100g^{-1}$)	Laurus nobilis	Ocimum basilicum	Origanum vulgare	Petroselium crispum
Caffeic acid	8.164	-	35.859	5.172
Chlorogenic acid	-	5.037	16.110	-

Phenolic compounds (mg 100g^{-1})	Laurus nobilis	Ocimum basilicum	Origanum vulgare	Petroselium crispum
Catechin	6.939	5.856	-	-
Ferulic acid	unq	-	32.222	-
Gallic acid	-	unq	11.500	40.276
m-coumaric acid	-	-	-	-
o- coumaric acid	-	-	-	-
p- coumaric acid	1.113	1.155	5.143	10.154
Resveratrol	-	-	unq	-
Trans-cinnamic	-	23.965	7.069	-
Vanillic acid	2.812	1.198	1.710	1.691
∑ Phenolic compounds	19.028	37.211	109.513	57.293

unq = unquantified (identified, but below the limit of quantification)
Source: Research Data.

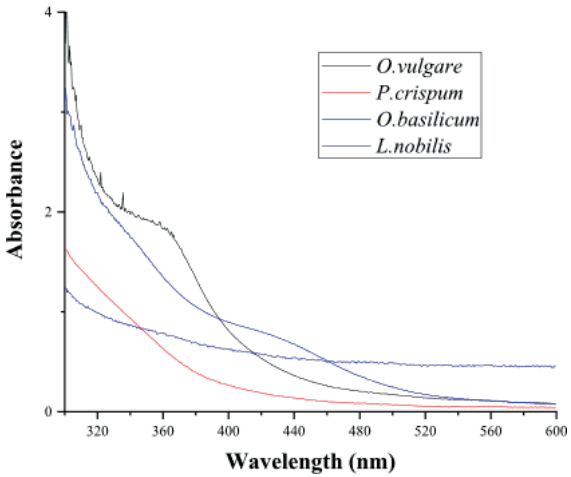
The ferulic acid found in the extract of *O. vulgare* has been described with multiple functions such as antioxidant, anti-inflammatory, inhibiting platelet aggregation and substances similar to thromboxane with inhibitory action on the formation of thrombi (LI *et al.*, 2021). Gallic acid was found in the extracts of *O. vulgare* and *P. crispum* in expressive concentrations. This compound was previously described with anti-inflammatory, antioxidant (NOURI *et al.*, 2020) and antimicrobial activity (RASOOLY *et al.*, 2020). Trans-cinnamic acid presented great potential in the wound healing process (VIANA *et al.*, 2020) and was found, in the present study in significant amount in the extracts of *O. vulgare* and *O. basilicum.* The *p*-coumaric acid was identified in all extracts and its use has been described for the treatment of cardiovascular, neuroinflammatory and liver diaseases (ABOTALEB *et al.*, 2020).

In this context, extracts of aromatic plants containing a diversity of actives compounds previously characterized and their association with probiotic bacteria were analyzed, in the present study, aiming to evaluate its effects on different activities induced by the main classes of enzymes present in the snake venom of the species *B. moojeni*, phospholipases A_2 (PLA_2s) and proteases.

3.2 ANTI-INFLAMMATORY ACTIVITY

The wavelength of hemoglobin absorption is 540 nm, and is released and measured in the liquid hemolysis test for evaluation of anti-inflammatory potential, whereas protection of the membrane against lysis is part of the anti-inflammatory actions. A scan was made in the extracts to investigate possible interferences in the evaluation of the activity. No peak was identified at 540 nm wavelength, leading to the conclusion that the extracts do not absorb in this wavelength (Figure 1).

Figure 1 – Spectrophotometer scans of different aromatic plant extracts (100 µg/µL): black- *Origanum vulgare*; red- *Petroselium crispum*; blue- *Ocimum basilicum*; purple- *Laurus nobilis.*



Source: Research Data

The extracts of *L. nobilis*, *O. basilicum, O. vulgare,* and *P. crispum* showed significant anti-inflammatory *in vitro* potential when compared with prednisolone and mechanical (C-) hemolysis control. Samples containing the extract of *P. crispum* presented a high protective effect on membranes lysis with heat-induced hemolysis percentages of 2.26 % and 1.53 % in absence of probiotics and, 0.98 % and 0.82 % in the presence of probiotics (1.75 and 3.5 mg doses, respectively). For the extract of **O.** *basilicum* evaluated in absence of probiotics, heat-induced hemolysis percentage was 7.39 % and 8.13 %, and in the presence of probiotics, the values of hemolysis (for 1.75 and 3.5 mg doses) were 4.75 % and 5.02 %, respectively.

The incubation with the extract of *L. nobilis* in absence of probiotics resulted in 12.22 % and 13.65 % of hemolysis and in the presence of probiotics with the previous doses the values were 10.03 % and 9.28 %. The extract of *O. vulgare* had the lowest inhibition potential of heat-induced hemolysis with results of 51.71 % and 55.18 % of hemolysis in absence of probiotics and 46.66 % and 52.72 % in presence of probiotics in the same doses of the extracts previously described (Table 2).

Table 2 – Anti-inflammatory potential evaluated on heating-induced erythrocyte lysis (54 ºC)

Controls	Hemolysis %
C- (PBS)	8.15 ± 1.5147
Probiotic ($9.6x10^5$ CFU)	6.89 ± 0.3084
Prednisolone (126 µg)	6.73 ± 0.4661

Controls	Hemolysis %	
Prednisolone (180 µg)	5.63 ± 0.6729	
C+ (H_2O)	100 ± 0.0087	
Samples	1.75 mg	3.5 mg
Laurus nobilis	12.22 ± 1.4255*[b]	13.65 ± 0.3814*a,b
Laurus nobilis+Probiotic	10.03 ± 0.8586[b]	9.28 ± 0.8743[b]
Ocimum basilicum	7.39 ± 0.3125[b]	8.13 ± 0.7020[b]
Ocimum basilicum+Probiotic	4.75 ± 0.8060[b]	5.02 ± 0.1578[b]
Origanum vulgare	51.71 ± 1.1919*[a,b]	55.18 ± 1.8249*[a,b]
Origanum vulgare+Probiotic	46.66 ± 2.5233*[a,b]	52.72 ± 2.5065*[a,b]
Petroselium crispum	2.26 ± 0.7237[a,b]	1.53 ± 0.6545*[a,b]
Petroselium crispum+Probiotic	0.98 ± 0.8460*[a,b]	0.82 ± 0.7150*[a,b]

a - It differs from the negative control (C-), $p < 0.05$.
b - It differs from the positive control (C+), $p < 0.05$.
*Statistically different from the drug, $p < 0.05$.
Source: Research Data.

3.3 PHOSPHOLIPASE AND HEMOLYSIS ACTIVITY

In the present work, alterations in the activity of PLA_2s were observed after incubation of *B. moojeni* venom with extracts of aromatic plants and probiotic bacteria. The extract of *L. nobilis* in pH 8.0 (Figure 2B), exerted the greatest inhibition on the activity of PLA_2s than in pH 7.4 (Figure 2A) at doses of 0.4, 1.8 and 2.5 mg with and without probiotic (values from 20 % to 25 %) (Figure 2B). The extracts of *O. basilicum* had significant inhibitions at pH 7.4 and pH 8.0 in all evaluated doses, with and without probiotics, with inhibitions values from 15 % to 17 % (Figure 2C and 2D).

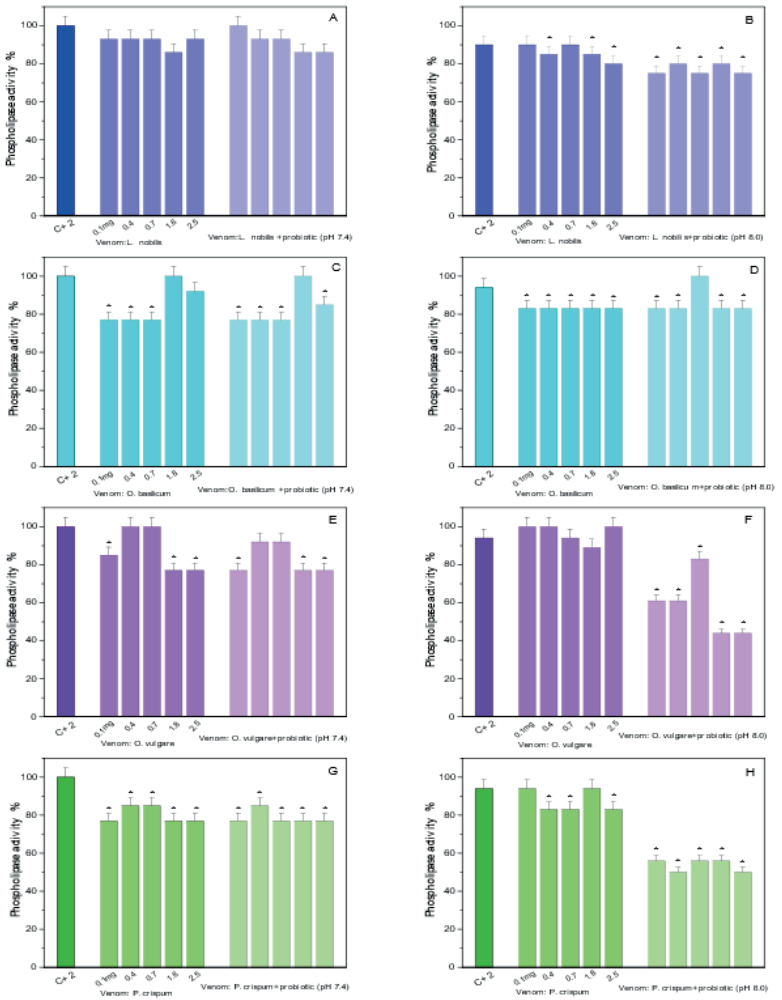
Origanum vulgare extract exerted the best inhibitions, around 23 %, when evaluated at pH 7.4 at doses of 1.8 and 2.5 mg, both in the absence and in the presence of probiotics (Figure 2E). For the evaluations at pH 8.0, in the absence of probiotics, there were no significant inhibitions and in the presence of probiotics, the greatest inhibitions were 39% at doses of 0.1 and 0.4 mg and 56 % at doses of 1.8 and 2.5 mg (Figure 2F).

The extracts of *P. crispum* at pH 7.4 in the presence and absence of probiotics, inhibited 15 % and 23 % at doses of 0.4, 0.7 mg, and 1.8, 2.5 mg, respectively (Figure 2G). At pH 8.0 the inhibitions in absence of probiotics were 17 % at doses of 0.4, 0.7 and 2.5 mg. In presence of probiotics, inhibitions were 44 % at doses of 0.1, 0.7, and 1.8 mg and 50 % at doses of 0.4 and 2.5 mg (Figure 2H).

In this research, Prednisolone and Dexamethasone at doses of 2.5, 5 and 10 µg were used for comparison to the effects caused by the extracts. The drugs did not show statistically significant

inhibitions compared to the control performed only with crude venom, considered as 100 % of the activity (data not shown).

Figure 2 – Phospholipase activity (%) induced by *Bothrops moojeni* venom, previously incubated with probiotic bacteria (pH 7.4 and pH 8.0) and extracts of *Laurus nobilis* (A,B), *Ocimum basilicum* (C,D), *Origanum vulgare* (E,F) and *Petroselium crispum* (G,H). Positive control 1 (C+ 1): containing only venom (10 µg) was considered as 100 % activity. Positive control 2 (C+ 2): Venom + Probiotic ($2.6x10^5$ CFU). The results correspond to the means of triplicates obtained in each proportion (venom: extract) and (venom: extract + probiotic) and their calculated standard deviations. *Statistically different from the positive control.

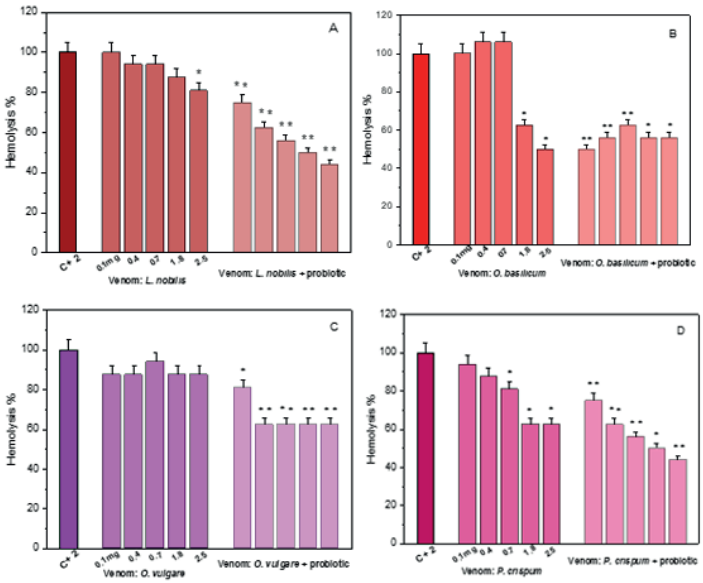


**Statistically different from the positive control and those incubated without probiotic.
Source: Research Data

In the present work, was observed the inhibition of 19 % on the hemolysis after incubation of the venom with 2.5 mg of *L. nobilis* extract in the absence of probiotics, and inhibitions between 25 % and 56 % for all doses evaluated in the presence of probiotics (Figure 3A). The extract of *O. basilicum*, showed significant inhibitions in absence of probiotics of 37.5 % and 50 % at doses of 1.8 and 2.5 mg, respectively. In the presence of probiotics, the inhibitions were 50 % at 0.1 mg, 37.5 % at 0.7 mg, and 44 % at 0.4, 1.8, and 2.5 mg (Figure 3B).

The extract of *O. vulgare* presented significant inhibition only in presence of probiotics, exerting 19 % inhibition at the dose of 0.1 mg and 37.5 % to the other doses evaluated (Figure 3C). The extract of *P. crispum* in the absence of probiotics inhibited the erythrocytes lysis in 19 % at the dose of 0.7 mg and 37.5 % at the dose of 1.8 and 2.5 mg. In presence of probiotics, the inhibitions were 25 % at 0.1 mg, 37.5 % at 0.4 mg, 44 % at 0.7 mg, 50 % at 1.8 mg and 56 % at 2.5 mg (Figure 3D). In this study, the probiotic bacteria and the extracts didn't induce hemolysis in any evaluated doses, except for *O. vulgare*, which showed hemolytic potential at the highest doses, 1.8 and 2.5 mg (data not shown).

Figure 3 – Hemolysis activity (%) induced by *Bothrops moojeni* venom, previously incubated with probiotic bacteria and extracts of *Laurus nobilis* (A), *Ocimum basilicum* (B), *Origanum vulgare* (C) and *Petroselium crispum* (D). Positive control 1 (C+ 1): containing only venom (20 µg) was considered as 100 % activity. Positive control 2 (C+ 2): Venom + Probiotic ($2.6x10^5$ CFU). The results correspond to the means of triplicates obtained in each proportion (venom: ex tract) and (venom: extract + probiotic) and their calculated standard deviations.

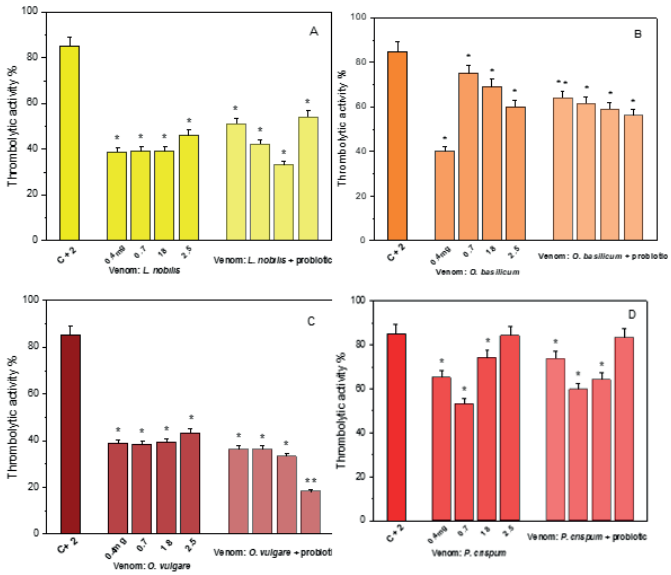


*Statistically different from the positive control.
**Statistically different from the positive control and those incubated without probiotic.
Source: Research Data.

3.4 THROMBOLYTIC AND COAGULANT ACTIVITY

The extract of *L. nobilis* inhibited the thrombolytic activity in percentages that varies from 46 % to 67 % in absence and presence of probiotics, in all evaluated doses (Figure 4A). *O. basilicum* extract inhibited thrombolytic activity at all doses evaluated in the absence and presence of probiotics, with values between 25 % and 44 % observed (Figure 4B). For the *O. vulgare* extract, the inhibition was significant both in the absence and in presence of probiotics at all evaluated doses, with values ranging from 57 % to 82 % (Figure 4C). For the extract of *P. crispum*, the inhibitions of thrombolytic activity were observed at the doses of 0.4, 0.7, and 1.8 mg with and without probiotics, the values ranging between 26 to 47 % (FIGURE 4D). Extracts of *L. nobilis, O. basilicum, P. crispum,* isolated probiotic bacteria and extracts associated with probiotic bacteria did not show significant thrombolytic activity. Except for the extract of *O. vulgare,* that showed 39 % and 22 % of thrombolytic activity when isolated and when associated with probiotic bacteria, respectively (data not shown).

Figure 4 – Thrombolytic activity (%) induced by *Bothrops moojeni* venom, previously incubated with probiotic bacteria and extracts of *Laurus nobilis* (A), *Ocimum basilicum* (B), *Origanum vulgare* (C) and *Petroselium crispum* (D). Positive control 1 (C+ 1): containing only blood (100 µL) + [venom (30 µg) in 30 µL of sample] was considered as 100 % activity, and therefore not shown in the graphs. Positive control 2 (C+ 2): Venom (30 µg) + Probiotic ($2.6x10^5$ CFU). The results correspond to the means of triplicates obtained in each proportion (venom: extract) and (venom: extract + probiotic) and their calculated standard deviations.



*Statistically different from the positive control.
**Statistically different from the positive control and those incubated without probiotic.
Source: Research Data.

The extracts of *L. nobilis, O. basilicum* and *O. vulgare* showed coagulation inhibition in all evaluated doses (in the absence and presence of probiotics). The extract of *P. crispum* showed coagulation activity in presence of probiotics in all evaluated doses. The coagulation inhibition was observed at doses of 0.1 and 0.7 mg and, pro-coagulation activity at doses of 0.4, 1.8 and 2.5 mg (Table 3).

Table 3 – Effect of aromatic plants extracts with and without probiotic on the coagulant activity induced by *Bothrops moojeni* venom

Clotting times (s)					
Controls					
Venom 10µg		127±0.0100			
Venom+Probiotic 2.6x10^5 CFU		77±0.0153 a			
Sample	mg	*Laurus nobilis*	*Ocimum basilicum*	*Origanum vulgare*	*Petroselium crispum*
Without Probiotic	0.1	186±0.0503 ^{b}	214±0.0305 ^{b}	246±0.0153 ^{b}	135±0.020 ^{b}
	0.4	209±0.2773 ^{b}	255±0.0462 ^{b}	268±0.0265 ^{b}	111±0.2715 ^{a}
	0.7	250±0.0283 ^{b}	247±0.0436 ^{b}	310±0.0608 ^{b}	132±0.0416 ^{b}
	1.8	254±0.0265 ^{b}	231±0.3782 ^{b}	319±0.0208 ^{b}	75±0.0265 ^{a}
	2.5	256±0.0231 ^{b}	213±0.4908 ^{b}	320±0.0305 ^{b}	74±0.0351 ^{a}
With Probiotic	0.1	254±0.0305 ^{b}	206±0.0305 ^{b}	151±0.0321 ^{b}	113±0.2524 ^{a}
	0.4	258±0.0603 ^{b}	223±0.2916 ^{b}	200±0.0208 ^{b}	91±0.0305 ^{a}
	0.7	190±0.2887 ^{b}	233±0.2639 ^{b}	247±0.0321 ^{b}	91±0.0361 ^{a}
	1.8	180±0.0666 ^{b}	252±0.1332 ^{b}	262±0.010 ^{b}	91±0.0321 ^{a}
	2.5	174±0.0351 ^{b}	263±0.010 ^{b}	304±0.0361 ^{b}	90±0.0379 ^{a}

a – Differ from the positive control ($p < 0.05$) – reduces time.
b – Differ from the positive control ($p < 0.05$) – increases time.
The results are presented as the average of triplicates ± standard deviation $p < 0.05$.
Source: Research Data.

4 DISCUSSION

Medicinal plants are known for their diversity of molecules with therapeutic activities with proven effectiveness against a wide variety of pathophysiological disorders. Isolated phytochemical molecules of several plant species have been described with action in reducing edema, inflammation,

myotoxicity, neurotoxicity and hemorrhage (URS *et al*., 2013, SANTHOSH *et al*., 2013). Several chemical compounds affect various enzymes in humans. These compounds exhibit therapeutic effects by altering the activities of enzymes drastically (ASLAN; BEYDEMIR, 2017).

Medicinal plant extracts have been studied for their potential anti-inflammatory action (LEELAPRAKASH; DASS, 2011) and the phenolic compounds present in these extracts are the main responsible for this action (YU *et al*., 2021b). The extract of the leaves of *L. nobilis* exerted an anti-inflammatory effect by reducing the expression of pro-inflammatory cytokines *in vitro* and *in vivo* (LEE *et al*., 2019). The extract of leaves of *O. basilicum* showed anti-inflammatory properties and, inhibit nitric oxide (NO) production showing the capacity to extinguish free radicals of NO (BENSAID *et al*., 2022), and the expressions interleukins (IL-6, IL-1) and chemosin ligant 2 decreased significantly when treated with the leaf extract (TAKEUCHI et al., 2020).

The extract of leaves of *O. vulgare* showed anti-inflammatory and antioxidant activities by inhibiting pro-inflammatory cytokines [IL-6 and tumor necrosis fator- (TNF-)] and NO without affecting cell viability (MIR *et al*., 2021). In the extract of the leaves of *P. crispum* was identified anti-inflammatory activity (FOUDAH *et al*., 2022) by inhibition of inflammatory cytokines (IL-1 and TNF-) through modulation of the oxidative stress which also increased the antioxidant activity of the extract (SOLIMAN *et al*., 2020).

For all these plant extracts described in the literature, the anti-inflammatory effect was attributed to phenolic compounds that represent a large part of their composition (HUANG *et al*., 2015). Some compounds present in these plant extracts (VLASE *et al*., 2014; KOLDAS *et al*., 2015; FERREIRA *et al*., 2022) were also found in the extracts of aromatic plants evaluated in the present work such as caffeic, chlorogenic, pherulic, gallic, *p*-coumaric, vanillic acids and catechin.

It is known that free radicals are important mediators that act by inducing and aggravating inflammatory process. Thus, free radical scavenger compounds present in the extracts reduce inflammation (DELAPORTE *et al*., 2002; GERONIKAKI; GAVALAS, 2006). Phenolic compounds have several properties such as antimicrobial, anti-inflammatory, antihyperglycemic, and antioxidant (YALTIRAK *et al*., 2009, PALANISAMY *et al*., 2011). Phenolic compounds, found in plants, are known to possess strong antioxidant properties and can capture free radicals, which leads to metal chelation, interactions with adenosine receptor enzymes, and biomembranes (SAIJA *et al*., 1995).

These compounds have been described as enzymatic modulators and have been shown to exert inhibitory or potentiating effects on several classes of enzymes. The literature suggests that these interactions are typically weak, and involve amino acid residues present in the active site of enzymes or residue regions that coordinate an ionic cofactor. Some phenolic compounds have also been shown to form complexes with cofactors that are unavailable to bind in enzymes. In the context of snake venom, inhibition of phospholipases A_2 (PLA_2s) and proteases by phenolic compounds plays a fundamental role in reducing the inflammatory response (SALES *et al*., 2017, CESAR *et al*., 2021).

In addition, strains of probiotic bacteria can exert beneficial effects on the host through their immunomodulatory activity and regulation of the inflammatory process. Probiotics administered orally can increase the activity of Natural Killer cells, the production of interferon (INF) and INF- (VILLENA

et al., 2013). The probiotic strain *Lactobacillus casei* acts in the elimination of free radicals (*in vitro*) and reduces oxidative damage, improving lipid metabolism and reducing lipid peroxidation (which can also be evaluated in vitro) (WANG *et al*., 2018).

Probiotics containing *Bifidobacterium, L. acidophilus, L. plantarum,* and *L. paracasei* decreased the levels of TNF- and IL-6 (HAJIFARAJI *et al*., 2018) and inhibit the nuclear factor kappa B (NF-kB) pathway (BANAN *et al*., 2007). The interaction between probiotic strains *Lactobacillus* and *Bifidobacterium* significantly reduced the expressions of IL-8, p-p65, NF-kB, p-p38 MAPK, vascular system adhesion molecule, and cyclooxygenase-2 (COX-2). The combination of probiotic strains showed better anti-inflammatory activity (LI *et al*., 2019). Considering the anti-inflammatory actions exerted by probiotics, evaluated *in vitro* and the effects described *in vivo*, we can suggest that probiotic supplementation together with the consumption of aromatic plants has potential as a preventive and protective adjuvant against the development of inflammation.

Steroid drugs are known as PLA_2s inhibitors. Although they are widely used in the treatment of inflammatory diseases, they have adverse effects such as accelerated protein loss, decreased protein synthesis, and bone demineralization (COHEN; SACHAR, 2017). Prednisolone is a glucocorticoid often used to suppress the immune response and treat inflammatory diseases (TISO *et al*., 2004). The most common side effects are fatigue, increased blood pressure and blood glucose, mental disorders, headaches, and its prolonged use can cause cataracts (GANBARJEDDI *et al*., 2020).

The synthetic anti-inflammatory drugs used in the treatment of inflammation are becoming less acceptable due to serious adverse effects on human health (XIAO *et al*., 2005) mainly due to their use in high doses, continuous or chronic. Treatment with these drugs in the long term has some effectiveness, but is associated which depression, growth retardation, osteoporosis, hypertension, kidney, and liver problems (TALLEY *et al*., 2011). The development of new and safe strategies to prevent and treat inflammatory diseases is of extreme medical and scientific relevance. Therefore, the extracts of *L. nobilis, O. basilicum, O. vulgare* and *P. crispum* showed anti-inflammatory potential and seem to be amplified by association with probiotics bacteria, since they also act in control of the inflammatory response (MARTYNIAK *et al*., 2021).

Venom is a complex mix of proteins and active peptides, including phospholipases A_2 (PLA_{2S}), metalloproteases (SVMPs), serine proteases (SVSPs), C-type lectins, L-animo acids oxidases, hyaluronidases and myotoxins (GUTIÉRREZ *et al*., 2017) that affect the hemostatic system, act on the inflammatory response, debridement and tissue regeneration, among other physiological effects (DAMEL *et al*., 1975) involved in hemolysis and cytolysis (DUFTON; HIDER, 1988). Snake venom is rich in PLA_2s enzymes. Those enzymes hydrolyze membrane phospholipids in position sn-2 (BURKE; DENNIS, 2009) and release lysophospholipids and fatty acids. The arachidonic acid leads to the production of pro-inflammatory mediators (eicosanoids) by the pathways of COX and lipooxygenases (CHAKRABORTI, 2003).

Inflammation is triggered by a complex biological response of body tissues extending to internal and external stimuli, which involve pathogens, and damaged or irritated cells. However, when there is an excess of inflammation, characterized by dilation of blood vessels, production of pro-inflammatory molecules, the release of cytokines, and recruitment of leukocytes, there can be serious damage and

losses to cells, tissues, and organs (SHIN *et al*., 2011). The inhibition of PLA_{2S} causes the decrease of eicosanoid levels and reduced inflammatory response (LATTIG *et al*., 2020). Many plants with anti-inflammatory potential are rich in phenolic compounds and some of these have been described as inhibitors of PLA_2s, such as extracts of grape bark, *Averrhoa carambola* and *Morinda citrifolia* (FREIRE *et al*., 2020, OLIVEIRA *et al*., 2021, MARQUES *et al*., 2021). In this way, wide research of the pharmacological effects exerted by various plants species on the different toxins classes can result in valuable knowledge that will allow their use, through food consumption or in the form of supplements, for promoting human health through the prevention and treatment of diseases.

In the present study, significant amounts of gallic acid were found in *O. vulgare* and *P. crispum* extracts Zhang *et al*. (2022) showed that gallic acid acts as a possible inhibitor of thrombin activity, whose catalytic action can be simulated *in vitro*, in the present study, using homologous proteases. Gallic acid is a chelator of metals (BADHANI *et al*., 2015) which justifies the inhibitory effect of the extracts of the present study on the activity of PLA_2s and hemolysis, partially induced by this class of enzymes, since these enzymes are dependent on Ca^{2+} ions to perform most of their functions.

In a study, the gallic acid inhibited 100 % of the hemolytic activity induced by *Bothrops jararaca* venom (PEREIRA Jr *et al*., 2022). The gallic acid was isolated from the aqueous extract of *Anacardium humile* and inhibited the activities of PLA_{2S} of the *B. jararacussu* venom and its PLA_{2s} isolated BthTX-II (COSTA *et al*., 2021). Quercetin-3-o-rhamnosideo, a compound isolated from *Euphorbia hirta* extract, inhibited the hemolysis activity induced by snake venom of the species *Naja naja*, suggesting inhibition of inflammatory activity, proteases and PLA_2s, *in vivo* (GOPI *et al*., 2016). The aqueous extract of the leaves of *Averrhoa carambola* inhibited the activity of PLA_2s from the venoms of *B. moojeni* and *B. alternatus*, and the same extract of *A. carambola* inhibited the hemolysis induced by the of *B. atrox* and *Crotalus durissus terrificus* (*C. d. t*) venom (OLIVEIRA *et al*., 2021).

The extract of *Eucalyptus urophylla* showed an inhibitory effect on proteases, being this effect attribute to phenolic compounds, terpenoids, alkaloids, and steroids (TREMACOLDI; PASCHOLATI, 2002). The aqueous extract of leaves of *Schawartiza brasilienis* inhibited the proteolysis induced by the venom of *B. jararaca* and *B. jararacussu*, besides inhibiting the hemolysis induced by the venom of *B. jararaca* (SOUZA *et al*., 2020). All these inhibitions described in the literature, for extracts from different plants acting on snake venoms of the *Bothrops* and *Crotalus* genera, corroborated the results of the present study, considering the rich phenolic composition attributed to plant extracts and the main classes of enzymes, potentially inhibited in the test carried out, focusing on PLA_2s and several proteases.

The various studies cited demonstrate that secondary metabolites act through different mechanisms, exerting synergistic action when we consider enzymatic modulations in the context of prevention and treatment of pathophysiological disorders.

Antioxidants present in plant extracts have protective effects against inflammation. Probiotics can stimulate the host's antioxidant system to improve antioxidant enzyme levels (SANDERS *et al*., 2019). According to Zeng *et al*. (2021), probiotics presented antioxidant potential and did not presented hemolytic activity. There is evidence that probiotics have valuable benefits in modulating inflammation (YAN *et al*., 2007), producing specific effects in healthy individuals (MEN *et al*., 2017). The probiotic bacteria most commonly used are *Bifidobacterium* and *Lactobacillus spp* (ZHANG *et al*., 2010).

Probiotic strains such as *Bifidobacterium, L. rhamnousus* and *L. acidophilus* regulate inflammation, producing anti-inflammatory effects (BORTHAKUR *et al.*, 2013). In addition to anti-inflammatory effects, immunomodulatory, antioxidant, and anti-lipidemic properties have been demonstrated for strains of *Bifidobacterium* (MEN *et al.*, 2017). The *in vitro* results obtained for the probiotics evaluated in the present work add to the scientific knowledge previously described for the effects of probiotics *in vivo*.

Considering the existing scientific information and the data from the present work, we can suggest that the reduction in hemolysis activity, observed after incubation of the venom with the different extracts of aromatic plants and probiotics, is the result of the inhibitory effect of the phenolic compounds present in the extracts and the metabolites produced by probiotic bacteria such as trimethylamine and short-chain fatty acids (LASSINGER-HERFURTH *et al.*, 2019) on PLA_2s and proteases present in the venom, as well as interactions between these natural compounds and components of cell membranes may also be associated with protective effects.

The polyphenols can act as complexes with metal ions, such as Ca^{2+} and Zn^{2+} for example, decreasing the activity of PLA_2s and proteases, that use metallic ions as cofactors in their catalytic activity (GOPI *et al.*, 2015), can still make hydrogen bonds, hydrophobic interactions, pi bonds between the nucleon of the compound present in the extracts and the proteins presents in the *B. moojeni* venom, reducing its catalytic activity (ANDERSEN; MARKHAM, 2005).

The *B. moojeni* venom is made up of 30 % to 60 % of SVMPs (CALVETE *et al.*, 2009). This SVMPs affect the hemostasis, inducing defibrinogenesis, activating coagulation cascade factor X and prothrombin (LIMA *et al.*, 2009). The SVMPs have the potential to act as thrombolytic agents and act as anticoagulants. The SVMPs have also the capacity to decrease fibrinogen levels and inhibit thrombogenesis (WANG *et al*. 2001). Equally important, the *B. moojeni* venom is made of 10 % to 30 % of SVSPs, being kallikrein-type enzymes that generate bradykinin with vasodilator and inflammatory function (BRAUD *et al.*, 2000).

The isolated action of enzymes present in bothropic venoms, such as PLA_2s, SVMP, and SVSP, as well as their joint action (SOUSA *et al.*, 2017), results in a series of disturbs in the hemostatic systems. Those toxins activate the coagulation system, consuming the coagulation factors, mainly fibrinogen. The fibrinogen is hydrolyzed in intravascular fibrin, which is also hydrolyzed, forming degradation products. This results in increased blood clotting time causing bleeding (SGRIGNOLLI *et al.*, 2011).

Corroborating the data of the present study, in which all the evaluated extracts exerted an inhibitory effect on the thrombolytic activity induced by the venom of *B. moojeni*, the extract of the leaves of *A. carambola* was described with the same action potential (OLIVEIRA *et al.*, 2021a). The aqueous extracts of *Aniba fragans* and *Philodendron megalophyllum* showed the potential to inhibit hemorrhages caused by *B. atrox* venom (GUIMARÃES *et al.*, 2020). Plants like *Diospyro discolor, Enobotrya deflez, Machulius japonica*, and *Pyros taiwanensis* showed high phenolic amounts in the chemical composition of their extracts also exert inhibition on the activity of the metalloproteases (LEE *et al.*, 2009).

The fact that plants are natural sources for inhibiting the SVMPs was noted in many research pappers, such as those that describe the *Euphoria longana* (PANYATHEP *et al.*, 2013)*, Macrocystis pyrifera, Camellia sinensis* and *Eucommia ulmoides* (ZALUSKI; SMOLARZ, 2009). The enzyme modulators

present in plant extracts belong to different classes of compounds, however, phenolics always stand out as the most abundant and responsible for the most varied mechanisms of action.

Corroborating the data of the present study, the gallic acid found in significant amounts in the extracts of *O. vulgare* and *P. crispum*, presented anticoagulant activity and inhibited the proteolytic and hemorrhagic activities induced by the venoms of *B. jararaca* and *B. jararacussu* (PEREIRA Jr *et al*., 2022). The extracts of *Schwartiza brasiliensis, Andrographis paniculata* and *Stryphnodendron adstringens*, rich in phenolic compounds, inhibited anticoagulant, proteolytic and hemorrhagic activities induced by *B. jararaca, Naja naja* and *B. jararacussu* venom (NAYAK *et al*., 2020, PEREIRA Jr *et al*., 2020). In the present study, *P. crispum* showed pro-coagulant activity, enhancing the action of enzymes present in the venom of *B. moojeni*, as well as the aqueous extract of the leaves of *A. carambola* showed pro-coagulant activity when evaluated after incubation with the venom of *C.d.t.* (OLIVEIRA *et al*., 2021b).

The presence of phenolic compounds in vegetables is correlated with beneficial effects on human health, the various biological actions have been attributed especially to phenolic acids (OLAS, 2019). The suggested mechanisms of action for these compounds are based on their ability to bind to metals such as Zn^{2+} and/or Ca^{2+} and macromolecules, and interaction with amino acid residues present in protein structures, thus blocking the pharmacological activities of enzymes (CARO *et al*., 2017).

For the most part, the drugs found in the pharmaceutical market were developed from compounds extracted mainly from plants. Based on studies found in the literature and on our results, the synergetic actions between the extracts of aromatic plants and their bioactive compounds with probiotic bacteria are promising in the treatment and prevention of diseases related to homeostatic and inflammatory disorders. The extract of *L. nobilis, O. basilicum, O. vulgare* and *P. crispum* exert modulating action on the activities induced mainly by PLA_2s and proteases, presenting anti-inflammatory, anti-hemolytic and anti-thrombolytic potential, in addition to interfering with the action of coagulant enzymes present in the venom used as a laboratory tool.

Additionally, studies suggest an improvement in the intestine condition through the administration of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (MATSUOKA *et al*., 2018). Probiotic bacteria release bioactive mediators that contribute to the maintenance of intestinal homeostasis and platelet aggregation without thrombotic tendency (ISOZAKI *et al*., 2021). During thrombus formation, various coagulation factors are involved in the action of thrombin, fibrinogen (FIB) is continuously transformed into fibrin, the main component of thrombus (WANG *et al*., 2016). Zeng *et al*. (2022) demonstrated that *L. plantarum* can control PT, APTT, TT and FIB, indicating interfering with thrombosis formation *in vivo*.

Platelet aggregation is an initial key event in thrombus formation. The *L. rhamnosus* and *Bifidobacterium lactis* are considered probiotic strains that don't participate of thrombotic disorders (ZHOU *et al*., 2005). Metabolites produced by probiotic strains dissolve thrombi and improve blood flow. Metabolites of *L. rhamnosus, L. casei,* and *L. plantarum* can modulate/regulate metalloproteases to prevent inflammatory diseases (YUE *et al*., 2020). Probiotic stains such as *L. casei* and *L. rhamnosus* are able to beneficially modulate the inflammation-coagulation interaction by reducing blood clotting activation (ZELAYA *et al*., 2014).

Although broad clinical/nutritional studies in humans still need to be developed, aiming to evaluate quantities of consumption, and associations between plant consumption and various other lifestyle factors, the large number of existing research proving pharmacological actions and mechanisms of action of the compounds present in aromatic plants, as well as the beneficial effects of various probiotics on the human body, are enough to recommend the addition of these natural compounds to the human food routine and/or supplementation, aiming above all at the prevention of chronic subclinical inflammations.

Therefore, understanding the action of extracts and probiotic bacteria on the modulation of hemostasis and the inflammatory response still requires further studies. However, due to the complexity of the composition of these extracts and the pathophysiological processes inherent to the human body, complementary scientific studies need to be carried out to elucidate possible mechanisms of action that are responsible for the benefits to human health, in addition to its association with several other lifestyle factors, which, influence human health.

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