In vitro anti-inflammatory and anti-cholesterolemic activities of *Chrysanthellum americanum* (L) Vatke harvested in Mali

ABSTRACT

Aims: The objective of this study was to evaluate the anti-inflammatory and anticholesterolemic activities of *Chrysanthellum americanum* extracts.
Place and Duration of Study: The plant material was harvested in September 2021 at Findiala located in Kolondièba, Mali. The phytochemical screening and evaluation of anti-inflammatory and anticholesterolemic activities were performed at the Laboratory of Plant and Food Biochemistry and Biotechnologies of the University of Sciences, Techniques and Technologies of Bamako (Mali) from September 2021 to January 2022.
Methodology: The phytochemical screening based on classical methods was performed. The *in vitro* anti-inflammatory activity was determined by the protein denaturation method. The binding capacity of protein hydrolysates to bile salts was used to evaluate the anticholesterolemic activity.
Results: The screening of *C. americanum* extracts revealed the presence of numerous secondary metabolites including alkaloids, polyphenols, and flavonoids. These extracts at different concentrations showed a good anti-inflammatory activity *in vitro*. Indeed, at 1 mg/mL of extracts with protein denaturation inhibition rates ranging from 73.33% to 79.42% respectively for infusion and decoction, against 93.33% for diclofenac (positive control). Cholesterol reduction rates ranging from 36.28±2.44% to 72.37±2.29% were recorded. Trypsin hydrolysates showed the best results with 72.37% binding to sodium cholate after 90 minutes followed by the decoction with 58.30% binding to sodium deoxycholate after 90 minutes of hydrolysis with infusion.
Conclusion: The data obtained from this study show the richness in anti-inflammatory and anticholesterolemic activities of *C. americanum* extracts. Therefore, this species could be a promising source of anti-inflammatory and anticholesterolemic agents.

Keywords: Chrysanthellum americanum, Anti-inflammatory, Anticholesterolemic, Salts biliary.

1. INTRODUCTION

The bioactive molecules from plants, due to their multiple biological activities, are currently of particular interest in the health, agri-food, cosmetic and pharmaceutical fields [1]. Nowadays, the non-transmissible diseases are the leading cause of death in the world. Among the 57 million deaths in 2008, 36 million, i.e. almost two thirds, were caused by these diseases, including cardiovascular, cancer, diabetes and chronic respiratory diseases [2], [3]. In addition, the increase in cholesterol levels (hypercholesterolemia) has become an important health problem in recent years. Hypercholesterolemia is known to be a risk factor for the development of cardiovascular diseases, including atherosclerosis, myocardial infarction and cerebral paralysis [4]. The dietary factors, such as the continuous ingestion of large amounts of saturated fat and cholesterol, are considered to be directly related to hypercholesterolemia [5]. The edible medicinal plants with antioxidant and anti-inflammatory capabilities could play a vital role in the management of high level of cholesterol [6].

The inflammation is a complex process, which is frequently associated with pain and involves events such as increasing of vascular permeability, increasing of protein denaturation and membrane alterations [7]. The aggressions are of various natures. They can be traumatic (cut, crush, sprain, fracture...), chemical or physical (burn, frostbite, ionizing radiation...), infectious origin (bacterial, viral, fungal, parasitic infections...). They can also due to endogenous or exogenous solid elements (urate crystals, toxins...) or of allergic origin [8]. However, when the inflammation is not controlled, it could cause the tissue destruction and a series of reactions, rousing the pain [9]. The most commonly used treatments are non-steroidal anti-inflammatory drugs (NSAID) and glucocorticoids [10], [11]. As a result, the research for more effective anti-inflammatory agents continues to be an area of interest that has led to an increase in the investigation for natural products with anti-inflammatory activity and no or fewer side effects. For several years, new anti-inflammatory and analgesic drugs with no or fewer adverse effects have been researched worldwide as alternatives to NSAID and opiates [10].

Chrysanthellum americanum (L) Vatke is very coveted for its medicinal properties in several West African countries. *Chrysanthellum americanum* (L) Vatke is a small, tropical, herbaceous and aromatic plant belonging to the Asteraceae family. This plant, widespread in the highlands of Africa, is native of South America. Its nutritional richness in flavonoids and saponosides gives it a beneficial action on the circulatory system. It relieves heavy legs and helps to relieve the liver after certain food poisoning [12]. The species is praised due to its richness in peptides and others bioactive molecules. In fact, the peptides derived from *in vivo* digestion of whole proteins by hydrolysis with bacterial proteases before ingestion have been reported to have specific bioactivities. Consequently, the synthesis of these bioactive peptides have received considerable attention in recent years [13], [14].

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It has been reported that cholesterol-reducing peptides inhibit the micellar solubility of cholesterol, thereby decreasing the *in vivo* absorption of cholesterol [15]. The biliary salts are the major metabolites of cholesterol and facilitate its elimination in the faeces through the formation of micelles that solubilize cholesterol in the bile. Thus, the depletion of biliary salts in the circulation would promote the conversion of cholesterol to additional biliary salts, resulting in a significant reduction of LDL cholesterol levels in the liver and serum [16].

The present work was undertaken to determine the best enzyme, capable to hydrolyze the protein from aqueous extracts of *C. americanum* through the binding capacity of peptides to biliary salts. The inhibitory activity of protein denaturation by the aqueous extracts of *C. americanum* at different concentrations was also studied to evaluate the anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1 Materials

The plant material was consisted of the whole part of *C. americanum* (Figure 1) collected at Findiala located in Kolondièba circle, Mali.

Figure 1. Picture of *Chrysanthellum americanum* (L) Vatke

2.2. Methods

2.2.1 Preparation of extracts

For the infusion, 25 g of powder were placed in 250 mL of boiling water at room temperature (30 °C). After 5 min, the filtrate was collected and reduced under pressure at 45 °C using an evaporator.

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For the decoction, 25 g of powder were dissolved in 250 mL of distilled water. The mixture was boiled for 5 min and then filtered after cooling. Thus, the recovered filtrate was also reduced under pressure using an evaporator.

2.2.2 Phytochemical screening

The characterization of the main chemical groups of different extracts was carried out using classical methods based on staining and precipitation reactions with specific chemical reagents [17], [18].

2.2.3 *In vitro* evaluation of anti-inflammatory activity

Protein denaturation was performed according to the method described by Gambhire et al. [19]. The reaction mixture consisted of 1 mL of Bovine Albumin Solution (BAS), 3 mL of phosphate buffered saline (PBS, pH 6.4) and 1 mL of extracts at different concentrations.

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After incubation at 37°C for 15 min, the mixture was heated to 70°C for 5 min. After cooling, the absorbance was measured at 660 nm against a blank made with distilled water.
The Diclofenac sodium was used as a positive control.

The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.2.4. *In vitro* evaluation of anti-cholesterolemic activity

2.2.4.1. Preparation and hydrolysis of defatted proteins

The protein hydrolysates from *C. americanum* were prepared according to the slightly modified protocol [20]. The defatted protein isolates were prepared by alkaline extraction at pH11.50, followed by precipitation at pH4.50 and then lyophilized. The 5% lyophilized defatted proteins in distilled water were subjected to enzymatic hydrolysis for 90 min by keeping the pH constant by adding 0.5M NaOH solution. Every 30 min, a volume of the hydrolysate was taken and heated at 85 °C for 10 min to inactivate the enzyme.

2.2.4.1 Bile salt binding assay

The *In vitro* binding capacity of hydrolysates to bile salts was determined according to the method described by Yoshie-Stark et al. [21]. Each bile salt (as substrate) was dissolved in 50 mmol/L of phosphate buffer (pH6.5) to obtain a 2 mM bile salt solution, thus corresponding to the physiological concentration of bile salt (1.5-7 Mm).

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2.3 Data analysis

The data analysis was done by Excel 2013 and Minitab 18.1 software. The Fischer test was used to compare the means obtained at the 0.05 threshold.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

The results of the phytochemical screening are shown in the Table 1.

Table 1. Characterization of secondary metabolites

Chemical Groups	Extracts	
	Decoction	Infusion

Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Coumarins	+	+
Triterpenes	+	+
Saponosides	+	+

*(+) Presence (-) Absence

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The phytochemical screening revealed the presence of many groups of secondary metabolites such as alkaloids, phenolic compounds (flavonoids, tannins, coumarins, and saponins) and Terpenes. These results were confirmed by other researchers [22] [23], but Cissé et al. [24] noted the absence of alkaloids in their extract of the aqueous decoctate.

3.2 Anti-inflammatory activity

3.2.1 Effect of protein denaturation

The inhibitory effects of aqueous extracts and diclofenac sodium (positive control) at different concentrations on protein denaturation are presented in Figure 1.
The results in this figure show an inhibition of protein denaturation that varied between extracts and concentrations.

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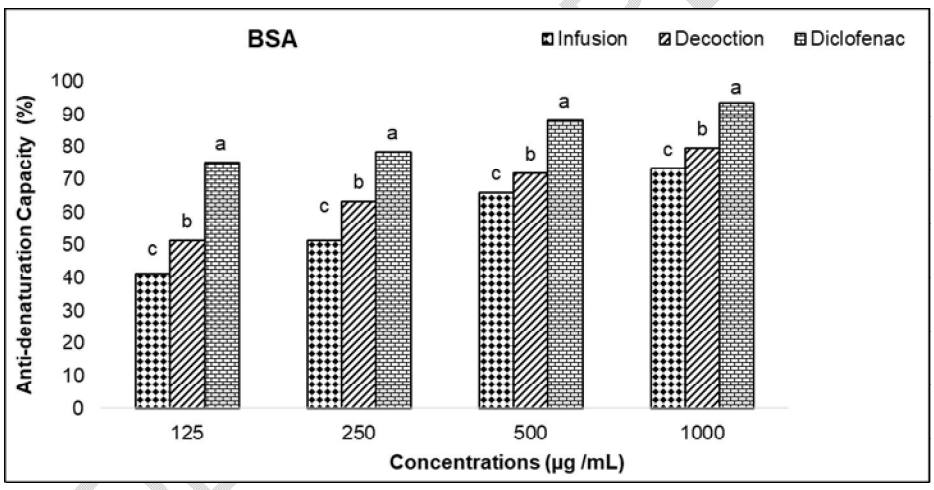

Figure 2. Effects of aqueous extracts and diclofenac sodium on protein denaturation.

*Different lower-case letters indicate significant differences ($p < .05$).

Table 2: Summary of percentage inhibition of protein denaturation for 1 mg/mL

Extracts	Infusion	Decoction	Diclofenac
Inhibition Level (%)	73.33%±1,61^{c}	79,42%±1,45^{b}	93,33%±1,53^{a}

The most biological proteins do not function properly when denatured and protein denaturation is one of the main reasons for inflammation [25]. In this research work, the infusion and decoction extracts of *C. americanum* showed inhibition of protein denaturation at concentrations from 125 µg/mL to 1000 µg/mL. The decoction with 1000 µg/mL showed the greatest inhibition rate of 79.42±1.45% which was lower than those of Diclofenac sodium (standard drug) with 93.33±1.53%.

This anti-inflammation power of *C. americanum* extract could be due to the presence of bioactive compounds including alkaloids, flavonoids, saponins, tannins, terpenoids and phenolic compounds [26]. Indeed, Yui et al. [27] reported that the alkaloids would inhibit TNF- α production either by inhibiting protein synthesis or by altering cysteine/methionine incorporation in macrophages [27]. Flavonoids such as hesperidin, luteolin, and quercetin are known for their anti-inflammatory properties. They act by affecting the enzymatic systems involved in the generation of inflammatory processes [28]. The anti-inflammatory activity of saponins is thought to be due to the inhibition of inflammation mediators such as histamine, serotonin and prostaglandin as well as its antioxidant property which inhibits the formation of the reactive oxygen species (ROS) which also plays a major role in inflammation [29], [30]. The anti-inflammatory effect of terpenoids is thought to derive from the inhibition of two enzymes in the inflammatory pathway of prostaglandins [31].

These compounds from *C. americanum* would be able to combat the oxidative stress and inhibit the action of enzymes responsible for the degradation and increase of sugar in a patient [32], [33].

3.3. Anticholesterolemic activity

3.3.1. Enzymatic hydrolysis of *C. amerianum* proteins by three protein enzymes:

In the present study, *C. amerianum* proteins were hydrolyzed independently for 90 min with alkalase, trypsin, and protease to determine the most appropriate enzyme for the production of bile salt binding peptides.

3.3.2. *In vitro* binding capacity:

Two bile salts were used to test the in vitro binding capacity of the different hydrolysates against that of atorvastatin.

3.3.3 Binding capacity of sodium cholate:

The Figure 3 represents the binding capacity of our infusion and decoction hydrolysates to sodium cholate.

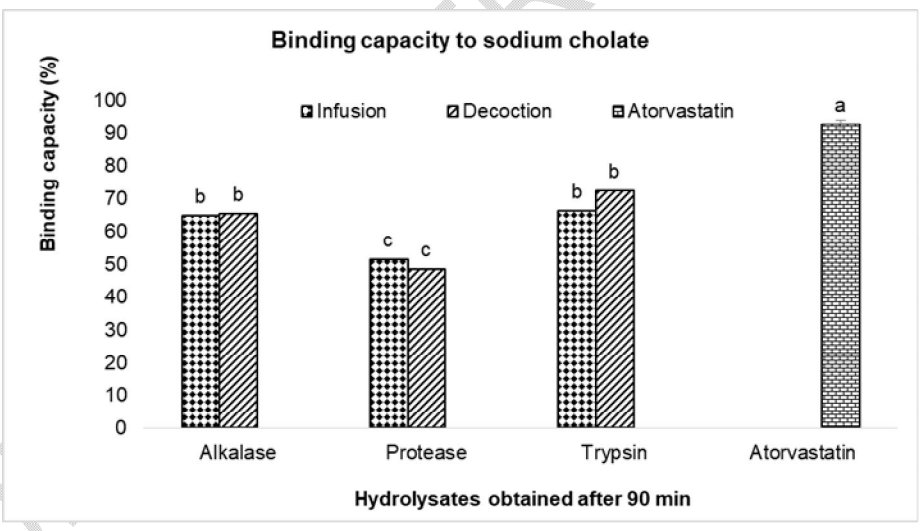

Figure 3. Binding of sodium cholate by peptides from the hydrolysates of *C. americanum*

**Different letters indicate significant differences ($p < .05$).*

3.3.4. Binding capacity of sodium deoxycholate

Under similar conditions, all *C. americanum* protein hydrolysates were hydrolyzed by enzymes: alkalase, trypsin, and protease. The Figure 4 represents the binding capacity of our infusion and decoction hydrolysates to sodium deoxycholate.

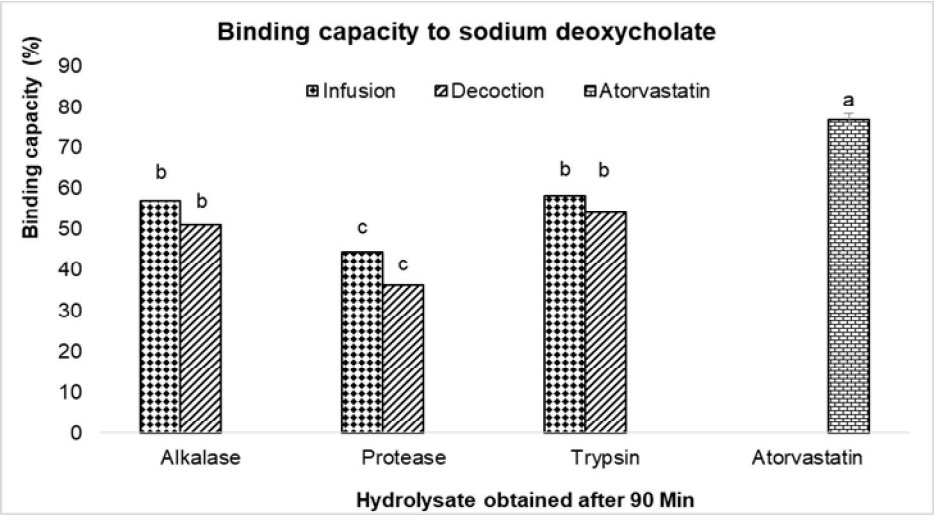

Figure 4. Binding of sodium deoxycholate by peptides from the hydrolysates of C. americanum

** Different letters indicate significant differences (p < .05).*

The sodium cholate was bound by all of the hydrolysates at different levels, from 48.39±2.86% for the 90 min protease hydrolysate to 72.37±2.29% for the 90 min trypsin hydrolysate; whereas Atorvastatin, used as a positive control, had a cholate binding capacity of 92.61±1.37% (Figure 3). The trypsin hydrolysate showed the highest cholate binding capacity (72.37%). Some previous studies [34]-[36] have indicated a cholate binding to hydrolysates of Guinea sorrel seeds (33.25%), defatted corn (9.99%), raisins (15-20%), wheat bran (10%), and potato peels (1.9-8.1%), respectively. The trypsin hydrolysate exhibited a higher cholate binding capacity than Guinea sorrel, defatted corn seeds.

Under the same conditions, all the protease-hydrolyzed protein hydrolysates of *C. americanum* showed a percentage of binding to sodium deoxycholate. These values were lower than those recorded with sodium cholate. The sodium deoxycholate was bound by 90 min protease hydrolysate from 36.28±2.44% to 58.30±3.38 by 90 min trypsin hydrolysate and 76.88±1.49 by atorvastatin.

The trypsin hydrolysate had the highest binding capacity compared to the other hydrolysates.
Our obtained results are lower than those of Kongo-Dia-Moukala et al. [35] who have reported 86.90% by Flavourzyme hydrolysate, but higher those mentioned by Tounkara et al. [34] with 48.28±6.71% and Camire and Dougherty [36] with 18.9%. Compared to these samples, our trypsin hydrolysates showed a greater binding capacity to sodium cholate.

The method reported here appears to be a satisfactory technique for measuring the binding capacity of peptides to bile salts. Atorvastatin, as expected, bound all selected bile salts under the same treatment. As the results showed, the hydrolysate obtained with trypsin contained effective peptides capable of binding bile salts. This could be explained by the presence of trypsin acting as an endoprotease. The higher binding of bile salts by the trypsin hydrolysate in our studies may be due to the use of a physiological pH6.5; which is closer to the pH of hydrophobic amino acids.

4. CONCLUSION

The present study shows that the extracts of *C. americanum* are rich in secondary metabolites. All investigated extracts of *C. americanum* possess anti-inflammatory and anticholesterolemic activities. Quite high protein denaturation inhibition rates were observed with all extracts. The decocted extracts showed the highest anti-inflammatory activity. Similarly, these extracts were able to bind all the bile salts tested. The best binding potentials were recorded with the trypsin hydrolysates of the decocted extract. These experimental data show that this species would be a potential source of anti-

inflammatory molecules and cholesterol-lowering agents for hypercholesterolemic patients. However, further study would be required to isolate the individual peptides responsible for the cholesterol-reducing activity of the hydrolysates and to identify their amino acid sequences in order to better elucidate the structure-function relationship of the peptides.

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