



## RESEARCH ARTICLE

## ANTIOXIDANT, ANTI-INFLAMMATORY, ANTIDIABETIC, ANTHELMINTIC, AND THROMBOLYTIC ACTIVITY OF THE METHANOL EXTRACT OF *CITRULLUS LANATUS* PEEL

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## Article Info:



## Article History:

Received: 3 February 2024

Reviewed: 12 March 2024

Accepted: 20 April 2024

Published: 15 May 2024

## Cite this article:

Nahar N, Fatema UK, Sharma PP, Bhuiya AM, Begum T, Azahar NF, Rahman MNA, Wan Sulaiman WMAB, Azad AK. Antioxidant, anti-inflammatory, antidiabetic, anthelmintic and thrombolytic activity of the methanol extract of *Citrullus lanatus* peel. Universal Journal of Pharmaceutical Research 2024; 9(2): 7-14.  
<http://doi.org/10.22270/ujpr.v9i2.1085>

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## Abstract

**Background:** The present study aimed for *in vitro* evaluation of *Citrullus lanatus* Peel (CLP) methanol extract for antioxidative, anti-inflammatory, antidiabetic, anthelmintic, and thrombolytic activity.

**Method:** CLP was extracted with methanol and tested for *in vitro* antioxidative (DPPH and H<sub>2</sub>O<sub>2</sub> scavenging method), thrombolytic, anthelmintic, antidiabetic, and anti-inflammatory properties (human red blood cell membrane stabilization method,  $\alpha$ -amylase inhibitory effect by using Iodine-Starch method).

**Results:** The percentage yield was 7% and phytochemical profile showed the presence of steroid, tannins, saponins, flavonoids, glycoside and reducing sugar. In DPPH scavenging assay, 88.7% was at 1000 g/ml, whereas 34% to 48.9% (IC<sub>50</sub> 27.65  $\mu$ g/ml) in H<sub>2</sub>O<sub>2</sub> and from 61.8% to 95.2% (IC<sub>50</sub> value: 7  $\mu$ g/ml) in ASCOR. In *in vitro* anti-inflammatory activity, the maximum protection percentage was observed 58% and 44%. Consecutively, in anti-diabetic activity, IC<sub>50</sub> values of 1137.64  $\mu$ g/ml whereas reference (Acarbose) exhibited IC<sub>50</sub> at 671.71  $\mu$ g/ml. On the other hand, the inhibitory effect of Acarbose showed 57% and 98% respectively at 2000  $\mu$ g/ml. In earthworms, it considered as very potential for the helminthic infection and helminthiasis. A significant clot lysis activity of 44% was noticed when the clots were treated with 100  $\mu$ l of CLP extract (10 mg/ml) in *in-vitro* thrombolytic activity assay.

**Conclusions:** The findings of this study revealed that the peel possessed the antioxidant, anti-inflammatory, antidiabetic, anthelmintic and thrombolytic activities. These findings will be motivated to do further research on molecular level to finds into more depth which is considered as the limitation of this study.

**Keyword:** Anthelmintic, antioxidative, anti-inflammatory, antidiabetic, *Citrullus lanatus* Peel (CLP), thrombolytic.

## INTRODUCTION

Epidemiological studies stated that the bioactive fruit has beneficial therapeutic effects on humans such as antidiabetic, anticancer, antihypertensive, neuro-protective, anti-inflammatory, antioxidant, antimicrobial, antiviral<sup>1</sup>, stimulation of the immune system, cell detoxification, and anticonvulsant<sup>1</sup>. *Citrullus lanatus* (CL) is a fruit that belongs to the *Cucurbitaceae* family. Watermelon fruit is largely rich in carotenoids and contains up to 92.0% (v/w) of

water<sup>2</sup>. The leaves, roots, stems, and seeds of CL plants contain phenols, tannins, saponins, alkaloids, flavonoids, steroids, carotenoids, and glycosides. The watermelon seed is a brain tonic, while the fruit itself has several medicinal uses, including cooling, aphrodisiac, diuretic, expectorant, and digestive stimulant. Besides CL fruit flesh possesses high levels of beta-carotene and vitamin C, which confer antibacterial, anticancer, and antioxidant properties. The fruit when canned or making into juices produces large amounts of by-products (BPs) (peel, pomace, seeds, etc.) that can exceed 50% by mass of entire fruit.

Though fruit BPs are devalued, they have a higher nutritional value and bioactive content than the victuals' part, presenting different health benefits<sup>3</sup>. The rind, seeds, and peels of CL fruit are often overlooked portions that are said to provide health advantages. Overlooked portion peel of CL ethyl acetate extracts contains flavonoids, terpenoids, cardiac glycosides, phenols, carboxylic acid, quinones, and xanthoprotein. CL also contains minerals (nutrition) such as Iron (Fe), manganese (Mn), zinc (Zn), and magnesium (Mg). However, lower than 1.0 mg/Kg calcium (Ca), copper, (Cu), potassium (K), nickel (Ni), and Lead (Pb) were also observed which do not possess.

CLP seed regulates blood sugar levels by releasing insulin from the  $\beta$ -cell of the islet of Langerhans, and CLP are well recognized for their analgesic qualities. In contrast to the peels, which are higher in total polyphenols, the red flesh contains more concentrated flavonoids<sup>4</sup>. Regarding DPPH experiment, the peel had the maximum antioxidant activity, followed by the pulp, rind, and seed. Antioxidants are elements that inhibit and alleviate the damage instigated by free radicals by providing electrons from antioxidants to damage cells. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. However, when an imbalance occurs with excessive production of free radicals, life-threatening diseases occur (cancer, diabetes, and cardiovascular and neurodegenerative diseases) due to a decline of the mechanisms of defense and damage to cell membranes<sup>5</sup>. For instance, the membranes of erythrocytes and lysosomes are comparable.

Lysosomes oxidize the lipids in cell membranes, causing harm to tissue. Lysosomes may bind to the asteroid anti-inflammatory receptor, or Hsp 90, and inhibit it from binding steroidal anti-inflammatory medicines, which would exacerbate inflammation. Protease release is inhibited, and inflammatory responses are decreased by stabilizing the lysosome membrane. The ability of plant extract to inhibit haemolysis can be interpreted as proof of their potency against inflammation. Hesperidin and Diosmin are two examples of flavonoids that have an anti-inflammatory impact by preventing the formation of prostaglandins. Certain flavonoids, like quercetin, block the 5-LOX and COX-2 enzymes. Certain flavonoids, such as quercetin, rutin, myricetin, kaempferol, and fisetin, prevent mast cells from releasing histamine. Numerous kinase enzymes, including phosphatidylinositol kinase and tyrosine kinase, are known to be activated during the inflammatory process. Numerous studies have also shown that flavonoids can competitively inhibit protein kinase C enzymes<sup>6</sup>.

Diabetes mellitus (DM) causes increased production of free radicals or impaired antioxidant defense. The excessive production of free radicals' damages cellular proteins, membrane lipids, and nucleic acids, and leads to cell death. DM can be controlled by different approaches like scavenging free radicals or improving the antioxidant enzyme activities. Another approach to control postprandial glucose levels could be by inhibiting intestinal  $\alpha$ -glucosidase and pancreatic  $\alpha$ -amylase<sup>7</sup>. Evidence suggests that polyphenols resemble

insulin in their effects on glucose consumption. They also function as effective inhibitors of important enzymes, such as alpha-amylase and alpha-glucosidase, linked to type 2 diabetes and tissue lipid peroxidation<sup>8</sup>. In diseases like type 2 diabetes, oxidative stress results in decreased muscle glucose absorption, lipid oxidation, and endothelial cell damage or dysfunction. These processes activate platelets and raise the risk of thrombosis and cardiac complications. Reactive oxygen and nitrogen species (RONS), free radicals, and the development of oxidative stress led to hyperactivity or enhanced platelet adhesiveness. The first stages of platelet-related thrombogenesis are represented by damage to the endothelium of the arterial wall or by atheromatous plaque injury. After adhering to the injured endothelium, platelets physically alter before activating and degranulating. Following platelet activation, fibrinogen binds to receptors on the platelet surface, ultimately leading to the formation of a thrombus<sup>9</sup>. Tissue plasminogen activator (t-PA), alteplase, anistreplase, urokinase (UK), and streptokinase (SK) are examples of commonly used thrombolytic drugs. However, in Indian regions, urokinase and streptokinase are the top choices because they are more affordable and readily. However, adverse effects such as allergic reactions, systemic fibrinolysis, bleeding, and immunogenicity are caused by streptokinase and urokinase. Acute myocardial infarction, reperfusion rate, and frequent early occlusions can all be effectively treated with thrombolytic therapy with recombinant t-PA. The platelet-rich thrombi exhibit strong resistance to t-PA-induced lysis<sup>10</sup>. Polyphenols and other natural compounds have demonstrated antioxidant and anti-thrombotic potential. Antioxidants have the potential to decrease platelet hyperactivity by inhibiting several receptors on the surface of the platelet and removing free radicals that initiate platelet activation<sup>11</sup>.

The presence of helminths in the body can lead to morbidity and mortality due to several factors such as impaired nutritional status, altered cognitive functions, tissue responses, intestinal obstruction, and rectal prolapse. The highest concentrations of intestinal worms and schistosomes are typically found in school-aged children (including teenagers) and preschoolers. This causes stunted growth and decreases physical fitness in addition to memory and cognition impairments that result in scholastic shortcomings<sup>12</sup>. Polyphenols are known to exert anthelmintic effects by preventing helminth parasites from producing energy, decoupling oxidative phosphorylation, or attaching glycoproteins to the parasite's cuticle, all of which cause the parasite to die<sup>13</sup>. To increase consumer knowledge of the benefits of CLP, we, therefore, plan to conduct *in vitro* activity of CLP methanolic extract against oxidation, inflammation, diabetes, atherothrombosis, and helminthiasis.

## MATERIALS AND METHODS

### Plant sample collection

CL fruits were properly cleaned to remove any remaining sand or dust. The peel (skin and rind) was

obtained by carefully scraping off the pulp, and a chipping machine was used to break the peel into pieces. In a hot air oven, peel chips were dried in two stages: first, for five hours at 50°C, and then for ten hours at 40°C, until the final product's moisture content reached around 10%. The material that made it through an 80-mesh screen was saved, sealed in polyethylene bags, refrigerated at 40°C, and utilized in a subsequent experiment<sup>14</sup>.

#### Preparation of CLP methanol extract and % yield

After homogenizing 200 g of CLP from powder and 2 L of methanol (1:10, v/v), the mixture was put in a beaker and shaken for one hour at room temperature at 200 rpm using an orbital shaker (Sk-L 330-pro, UK). Next, CLP extract was filtered using Whatman No. 1 filter paper to remove the residue. After twice extracting the residue left behind, the two extracts were mixed. The residual solvent was eliminated using a rotary evaporator (VP-30, Lab Tech, China) at 45°C and reduced pressure.

#### Qualitative phytochemical screening

According to Junaid and Patil<sup>15</sup>, steroids, tannins, saponin, flavonoids, glycoside, and Fehling test were detected using qualitative phytochemical assays of CLP extracts. Steroids were detected with a few drops of strong sulfuric acid added to the mixture and a few milligrams of CLP dissolved in chloroform. The red color where two layers converge indicates the presence of steroids. Two milliliters of CLP extract and one milliliter of a solution created by combining equal parts Fehling's solution A and B were brought to a boil and left there for a few minutes in order to detect decreasing sugar. Brick red precipitation was found, indicating the presence of reducing sugar. As per Junaid and Patil<sup>15</sup>; qualitative phytochemical assays of CLP extracts were performed for the detection of steroids, tannins, saponin, flavonoids, glycoside, and Fehling test. A few milligrams of CLP dissolved in chloroform and a few drops of strong sulfuric acid added to the mixture allowed for the detection of steroids. The presence of steroids is confirmed by the red hue at the intersection of two layers. To identify decreasing sugar, two milliliters of CLP extract and one milliliter of a solution made by mixing equal parts Fehling's solution A and B were heated to a boil for several minutes. Precipitation turned out to be brick red in color, indicating the presence of reducing sugar. Tannins were found using a 5 ml solution of CLP extract in a test tube.

One milliliter of a 0.1% ferric chloride solution was then added. The presence of tannins is indicated by a greenish-black color precipitate. To find flavonoids, a tiny amount of CLP methanol extract was mixed with drops of strong HCl. Flavonoids are indicated by the rapid development of a red tint. To find out if saponins were present, a 20 ml extract solution was diluted with 19 ml of distilled water and agitated for 15 minutes within a graduated cylinder. Saponin is present when there is a 1 cm froth on the liquid layer. To identify glycosides, dissolve a small amount of CLP extract in one milliliter of distilled water and then add a few drops of an aqueous NaOH solution. The presence of glycosides is verified when a yellow tint develops<sup>16</sup>.

#### Antioxidative activity

##### DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay

The stability of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical was used to measure the antioxidant capability of the CLP methanol extract. A persistent free radical with an odd electron in its structure, DPPH is commonly used in chemical analysis to detect radical scavenging activity. Dosages of CLP extract of 250 µg/ml, 500 µg/ml, and 1000 µg/ml were combined with an aliquot in 3 milliliters of a 0.004% EtOH solution of DPPH. The absorbance at 517 nm was measured after 30 minutes, and the inhibitory concentration (IC<sub>50</sub>) was computed. The blank's composition was 80% (v/v) methanol. Ascorbic acid, or vitamin C, was used as a comparator. Three measurements were taken. The IC<sub>50</sub> value, as reported, indicates the sample concentration required to scavenge 50% of the DPPH free radicals<sup>16</sup>; The DPPH scavenging effect was calculated using the following formula:

$$\text{DPPH of scavenging effect (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

##### Hydrogen peroxide assay

A 40 mM hydrogen peroxide solution is added to a 50 mM phosphate buffer solution with a pH of 7.4. The hydrogen peroxide concentration is measured at 230 nm using UV spectrophotometer. Hydrogen peroxide is mixed with CLP extract at 250, 500, and 1000 µg/ml in distilled water. After ten minutes, absorbance at 230 nm is measured in comparison to a black solution that contains phosphate buffer but no hydrogen peroxide<sup>25</sup>. Methanol made up 80% (v/v) of the blank. For comparison, ascorbic acid, or vitamin C, was utilized. There were three measurements made. The concentration of sample needed to scavenge 50% of the hydrogen peroxide is indicated by the IC<sub>50</sub> value. The following formula was used to determine the percentage of hydrogen peroxide scavenging effect:

$$\text{Hydrogen peroxide of scavenging effect (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100$$

##### Anti-inflammatory activity

Human red blood cells (HRBCs) were used in the membrane stabilization test to evaluate the CLP extracts' anti-inflammatory qualities. The donor was a healthy adult female who had given up taking birth control pills and anti-inflammatory drugs two weeks prior to giving venous blood. Alsever's solution, consisting of 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water, was combined with blood in an equivalent volume. Following a ten-minute centrifugation at 3000 rpm for the mixture, the packed cells underwent three rounds of washing with an isosaline solution (0.9%, pH 7.2), with the supernatant being discarded. To generate the assay mixture, 1 mL of phosphate buffer (pH 7.4), 2 mL of hyposaline solution (0.36%), and 0.5 mL of HRBC suspension (10% v/v) were mixed with 1 mL of either plant CPL extract at 125, 250, and 500 µg/mL or the reference medication diclofenac sodium at 125, 250, and 500 µg/mL. Phosphate buffer was utilized as the blank and distilled water was used in place of the plant sample in the reaction mixture. After 30 minutes of incubation at 37°C, the mixtures were centrifuged at

3000 rpm. Using spectrophotometry, the amount of hemoglobin in the supernatant solution was measured at 560 nm. It was demonstrated that all hemolysis occurred when distilled water was present. The formula was used to determine the HRBC membrane's stability percentage<sup>17,18</sup>.

$$\% \text{ protection} = 100 - \frac{\text{OD sample}}{\text{OD control}} \times 100$$

#### Antidiabetic activity [ $\alpha$ amylase enzyme assay (Starch-Iodine color assay method)]

The starch iodine test was modified slightly and used to screen CLP extract for  $\alpha$ -amylase inhibitors. Test samples of different concentrations were mixed with 500  $\mu$ L of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mmol sodium chloride) containing 0.04 units of the  $\alpha$ -amylase solution. After that, the mixture was incubated for ten minutes at 37°C. Following that, 500  $\mu$ L of soluble starch (1%, w/v) was added to each reaction well, and it was incubated for 15 minutes at 37 °C. To stop the enzymatic process, 20  $\mu$ L of 1 M HCl was added. Next, 100  $\mu$ L of iodine reagent (which contains 5 mmol I<sub>2</sub> and 5 mmol KI) was added. The absorbance at 620 nm was measured with a microplate reader, and the color shift was noted<sup>28</sup>. It was possible to establish a control reaction that showed full enzyme activity. The inhibition of enzyme activity was calculated as follows:

$$\% \text{ inhibition} = 100 - \frac{(\text{O. D. of control} - \text{O. D. of test sample})}{\text{O. D. control}} \times 100$$

#### Thrombolytic activity

##### Streptokinase (SK)

Five milliliters of sterile distilled water were added and thoroughly mixed with the commercially supplied lyophilized SK vial (Incepta Pharmaceutical, Bangladesh) containing fifteen million I.U. For *in vitro* thrombolysis, 100  $\mu$ L (30,000 I.U.) of this solution was utilized as a stock<sup>19</sup>.

##### Specimen

Twenty healthy human volunteers (n = 20) without a history of oral contraceptive or anticoagulant medication had their whole blood (4 ml) extracted. To create clots, 500  $\mu$ L (0.5 ml) of blood was added to each of the three microcentrifuge tubes that had been previously weighed.

##### Herbal preparation

On a vortex mixer, 100 mg of methanol extract CLP was suspended in 10 ml of distilled water and agitated forcefully. The soluble supernatant was separated from the suspension by decanting it after it had been left overnight and passing it through a 0.22-micron syringe filter. To test for thrombolytic action, 100  $\mu$ L of this herb's aqueous solution was applied to the microcentrifuge tubes holding the clots. The clot lysis experiments were conducted as previously reported. Venous blood was extracted from healthy individuals and placed into separate, sterile, pre-weighed Alpine tubes (500  $\mu$ L each tube), which were then incubated for 45 minutes at 37°C. Serum was eliminated (aspirated out without disrupting the formed clot) after clot formation. To find the clot weight, each tube containing a clot was weighed once again.

$$\text{Clot weight} = (\text{weight of tube with clot} - \text{weight of tube alone}) \times 100$$

After accurately labeling each Alpine tube holding clot, 100  $\mu$ L of peel extract was applied. Following a 90-minute incubation period at 37°C, clot lysis was monitored in each tube. Water was utilized as a negative control (no thrombolysis) and streptokinase as a positive control. There were ten iterations of the experiment.

$$\% \text{ Clot lysis} = \frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \times 100$$

#### The anthelmintic activity

With a few minor adjustments, adult *Pherithema posthuma* earthworms were used for the anthelmintic activity. Since earthworms are anatomically and physiologically similar to human intestinal roundworm parasites, equal-sized earthworms were employed in all experimental regimens. Two groups of three adult earthworms each were created from the worms. Separate stock solutions (100 mg/20 ml, 200 mg/20 ml) of the methanolic extract and 200 mg tablets of albendazole were made by dissolving the respective components in distilled water. By extracting varying quantities from the stock solutions, concentrations of CLP 2.5, 5, and 10 mg/ml in normal saline were created. Earthworms were released in groups into the above-mentioned Petridis having the desired concentration. As the standard group, they were given albendazole pills and a methanol extract of CLP. The amount of time it took for each worm to become paralyzed and die was noted. The time at which the worm died (min) was recorded as the meantime for paralysis, which occurred when no movement of any kind was seen, except for violent shaking. Once it was determined that the worm did not move in response to external stimuli or shaking, it was recorded<sup>20</sup>.

#### Statistical analysis

The results are shown as mean  $\pm$  SEM. ANOVA was used to evaluate the data in one or both ways. Tukey's multiple comparison tests were then run (using IBM SPSS Statistics for Windows, Version 25.0, Armonk, N.Y., USA) to establish significance at  $p < 0.05$ .

## RESULTS

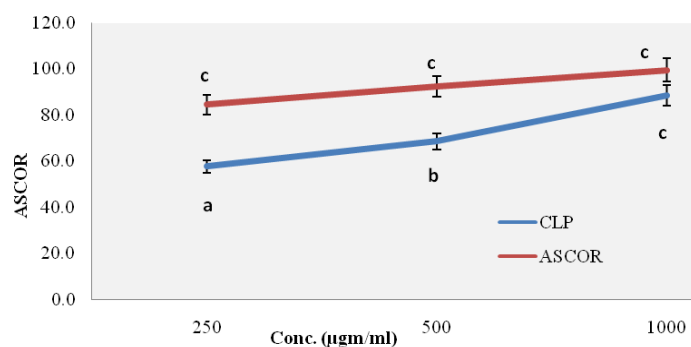
#### Percentage yield and phytochemical profile of CLP extraction

The percentage yield of CLP methanol extraction was 7% and the phytochemical profile showed the presence of steroid, tannins, saponins, flavonoids, glycoside and reducing sugar.

#### Assay for antioxidative activity of CLP

##### Antioxidative activity (DPPH)

The antioxidative activity of CLP extract was evaluated by means of the DPPH free radical scavenging method, and its scavenging activity was compared with that of ascorbic acid, the standard antioxidant. CLP extract showed a negligibly significant amount of DPPH free radical scavenging activity when compared to ascorbic acid. The concentration with the greatest scavenging action (88.7%) was 1000  $\mu$ g/ml out of the three (250, 500, and 1000 g/ml). It shows that ascorbic acid (ASCOR) showed activity at 250, 500, 1000, and  $\mu$ g/ml, respectively, of 84.5%, 92.5%, and 99.5%.



**Figure 1: The antioxidative of *C. lanatus* peel extract using DPPH method.**

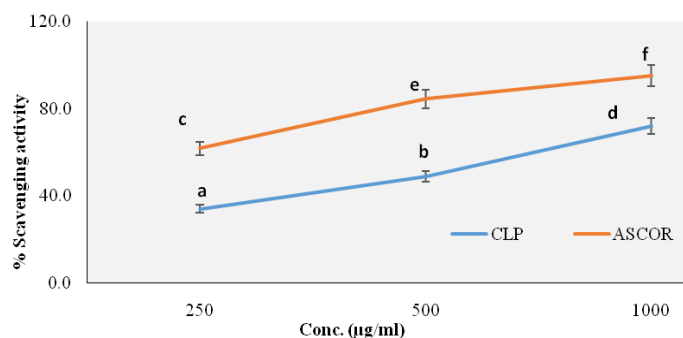
Data presented as mean  $\pm$  SEM, marked with similar letters (a, b, c, d, e, f) are not significantly different at  $p < 0.05$ , (n = 3), ASCOR= Ascorbic acid.

The  $IC_{50}$  (inhibition concentration 50) value was calculated using linear regression analysis, with the percent (%) inhibition or scavenging activity graphed against log concentration. The results showed that the  $IC_{50}$  values of ascorbic acid and CLP extract were 10.12  $\mu$ g/ml and 60.61  $\mu$ g/ml, respectively (Figure 1).

#### Antioxidative activity ( $H_2O_2$ )

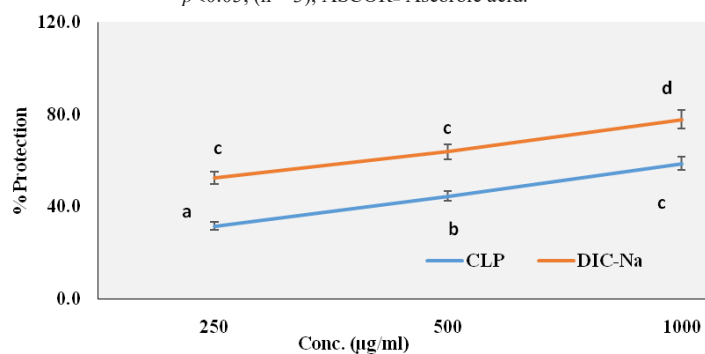
The CLP extract's ability to neutralize hydrogen peroxide ranged from 34% to 48.9% ( $IC_{50}$  value: 27.65  $\mu$ g/ml) and from 61.8% to 95.2% ( $IC_{50}$  value: 07  $\mu$ g/ml) in ASCOR. Hydrogen peroxide may be

neutralized by the CLP extract; however, this action was dose-dependent. Because hydrogen peroxide can pass across cellular membranes, it is significant. Although hydrogen peroxide doesn't react easily on its own, it occasionally poses a risk to cells due to its ability to produce hydroxyl radicals within them. The reason behind CLP's scavenging of hydrogen peroxide is its phenolic component, which can transfer electrons to hydrogen peroxide, neutralizing water in the process (Figure 2).



**Figure 2: The antioxidative activity of *C. lanatus* peel extract shows antioxidative activity using  $H_2O_2$  method.**

$p < 0.05$ , (n = 3), ASCOR= Ascorbic acid.



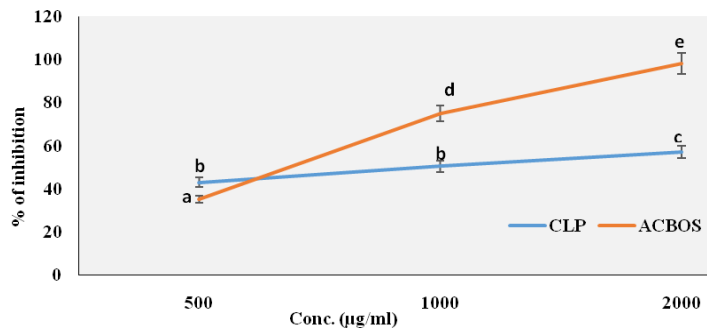
**Figure 3: The anti-inflammatory activity of *C. lanatus* peel extract.**

$p < 0.05$ , (n = 3), DIC-Na= Diclofenac Na.

#### In vitro anti-inflammatory activity

Lysosomal enzymes are released during inflammation, and a number of frequent alterations may occur. The lysosomal membrane's stabilization inhibits the release of chemical mediators and the lysosomal components of activated neutrophils, hence restricting the inflammatory response. This is how a lot of anti-inflammatory medications work. Since the membranes of erythrocytes and lysosomes are somewhat similar,

the stability of the erythrocyte membrane may be extrapolated to the lysosomal membrane. We have assessed how the CLP methanol extract affects the HRBC membrane's ability to stabilize. When CLP was extracted with methanol at 500 and 1000  $\mu$ g/mL, the maximum percentage of protection was observed 58% and 44%. However, compared to regular diclofenac sodium, CLP extracts showed reduced activity (Figure 3).



**Figure 4: The antidiabetic activity of *C. lanatus* peel extract.**

Data presented as mean  $\pm$  SEM, marked with similar letters (a, b, c, d, e) are not significantly different at  $p < 0.05$ , (n = 3), ACBOS= Acarbose.

### Anti-diabetic activity

This study investigated the  $\alpha$ -amylase inhibitory effects of the CLP compared to acarbose using the starch iodine color assay. At 500, 1000, and 2000  $\mu\text{g/ml}$  (43%, 50%, and 57%), CLP exhibits strong inhibition. At a concentration of 2000  $\mu\text{g/ml}$ , the standard medication acarbose demonstrated a very high level of inhibition of 98.18%, surpassing that of the CLP extract. Acarbose, the reference medication, and CLP extract had  $\text{IC}_{50}$  values of 671.71  $\mu\text{g/ml}$  and 1137.64  $\mu\text{g/ml}$ , respectively (Figure 4).

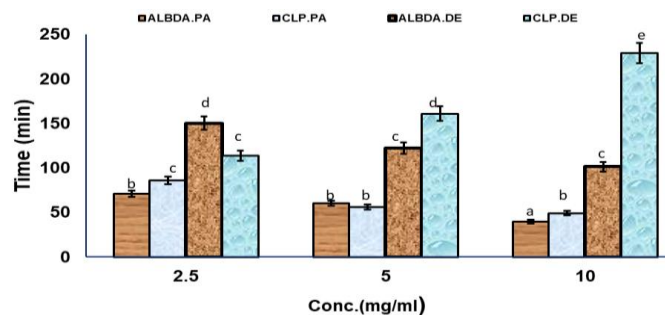
### In-vitro anthelmintic activity

In earthworms, CLP extracts exhibited anthelmintic action. The standard reference for anthelmintic activity evaluation was albendazole. The CLP extract's methanol extract at concentrations of 2.5, 5, and 10

mg/ml resulted in paralysis at 86, 56, and 49 minutes as well as death at 114 minutes, 161, 229 respectively. At 2.5, 5, and 10 mg/ml concentration of earthworm, standard albendazole treatment produced paralysis times of 71, 60, and 40 min, respectively, and death times of 114, 161, and 229 min, respectively. Finding the essential phytoconstituents would be crucial, as the methanol extract of CLP demonstrated action that was equivalent (Figure 5).

### In-vitro thrombolytic activity

Significant clot lysis activity of 44% was seen when the clots were treated with 100  $\mu\text{l}$  of CLP extract (10 mg/ml) concentrations. The results and mean *in-vitro* thrombolytic activity. Whereas, 100  $\mu\text{l}$  of Streptokinase, a positive control containing 30,000 IU, was added to the clots, 83% of the clots exhibited lysis in Figure 6.



**Figure 5: The anthelmintic activity of *C. lanatus* peel extract (CLP).**

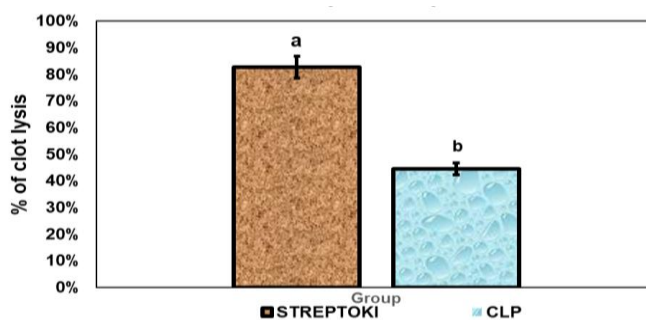
$p < 0.05$ , (n = 3), ALBDA. PA- Albendazole paralysis time, CLP. PA- *Citrullus lanatus* paralysis time, ALBDA.DE- Albendazole death time, CLP. DE- *Citrullus lanatus* death time.

## DISCUSSION

The pharmaceutical industry has looked to traditional medicine to find bio-active ingredients that can be combined with other ingredients to make synthetic medications. The most widely used species is what they wish to test. We study CLP extract in accordance with this hypothesis, which indicates the existence of flavonoids, glycosides, saponins, steroids, tannins, and polyphenols. The majority of the pharmacological effects of the CLP extract are attributed to these ingredients<sup>21</sup>. Antioxidants have the capacity to shield the body from oxidative stress brought on by free radicals. CLP's antioxidant activity for DPPH scavenging was compared to that of reference antioxidant, vitamin C. The phenol content of the CLP extract is consistent with the DPPH results that were

obtained<sup>22</sup>. Furthermore, the CLP extract scavenges hydrogen peroxide because it contains phenolic compounds that can give electrons to hydrogen peroxide, neutralizing it against water<sup>23</sup>.

Living tissues react to damage by becoming inflamed. To influence a longer-term response, steroids may cause lymphocytes to lyse and perhaps reallocate, which results in a quick drop in peripheral blood lymphocyte numbers. The balance of the lysosomal membrane is critical in reducing the inflammatory response because it inhibits the release of lysosomal components of activated neutrophils, such as bactericidal enzymes and proteases, which further exacerbate tissue inflammation and damage upon extracellular release. The findings showed that CLP extracts have strong anti-inflammatory properties at different doses<sup>24</sup>.



**Figure 6: Thrombolytic activity of *C. lanatus* peel extract.**  
 $p < 0.05$ , (n = 20), STREPTOKI=Streptokinase.

The alpha-amylase inhibiting action of CLP was demonstrated in methanolic extracts. Bioactive chemicals that limit enzyme activity are extracted by CLP; future research should focus on further elucidating the structure. The current investigation was limited to evaluating the CLP extract's potential as an enzyme inhibitor. Given that research links polyphenols to the antidiabetic effects of herbal extracts, flavanols or phenolic acids may be the expected bioactive components<sup>25</sup>.

Coagulation variables, in addition to other risk factors like aging, obesity, protein C deficiency, etc., influence the risk of venous thrombosis. One of the indicators for numerous vascular complications is oxidative stress, which is characterized as an imbalance between pro-oxidant and antioxidant systems. Because of its potential as an antioxidant, CLP has been shown to be effective in lowering oxidative stress. Certain characteristics of CLP extracts may aid in the prevention of vascular illnesses. The study's findings indicate that CLP extract has mild to moderate thrombolytic activity, which presents a chance to investigate its potential applications in the treatment of hypercoagulable states<sup>26</sup>. The secondary metabolites of CLP, tannin and alkaloid, are what give it its anthelmintic properties, according to a phytochemical test. Studies on the anthelmintic properties of flavonoids, alkaloids, and tannins are available. Tannins have been demonstrated to obstruct coupled oxidative phosphorylation, which prevents these parasites from synthesizing ATP.

Lastly, the *in vitro* techniques offer a quick way to check various plant extracts for possible anthelmintic properties. The results obtained using the *in vitro* approach could not be generalized to *in vivo* activity due to drug biotransformation, interactions with food components, and changes in absorption. Accordingly, *in vivo* evaluation should be used to determine the results<sup>27</sup>.

#### Limitations of the study

The main limitation of this study considered that this study performed *in vitro* only, in addition, the study applied crude extract, so there is more necessary to conduct isolation of the responsible bioactive compounds and recommending for *in vivo* study with isolated compounds in future for best outcome.

## CONCLUSION

In conclusion, more research is required to develop a potential and valuable antioxidative, anti-inflammatory, anti-diabetic, thrombolytic and anthelmintic therapy using of this plant origin.

## FUNDING

This research was supported by the TASREF Grant (UCMI/TNCP/RMC/Geran/2022(40)), University College MAIWP International, 68100 Kuala Lumpur, Malaysia.

## ACKNOWLEDGEMENT

The authors extend their gratitude to the Faculty of Pharmacy, USTC, Chittagong, Bangladesh and Faculty of Pharmacy, UCMI, Malaysia for supporting the research work by facilitating the necessary fund TASREF Grant (UCMI/TNCP/RMC/Geran/2022(40)).

## AUTHORS' CONTRIBUTIONS

**Nahar N:** conception and design of the work. **Fatema UK:** acquisition, analysis. **Sharma PP:** interpretation of data. **Bhuiya AM:** review, editing. **Azahar NF:** literature survey. **Rahman MNA:** lab works, editing. **Wan Sulaiman WMAB, Taslima Begum:** formal analysis. **Azad AK:** resources, writing of original manuscript. All authors have read and approved the final version of the manuscript

## DATA AVIABILITY

The data will be available to anyone upon request from the corresponding author.

## CONFLICT OF INTEREST

None to declare.

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