# Evaluation of In-vitro Anti-inflammatory, Thrombolytic and In-vivo Acute Toxicological Activity of *Camellia Chrysantha (Hu) Tuyama*


**ABSTRACT**

**Purpose:** The objective of this study was to investigate the impact of methanol-prepared leaves extract of*Camellia Chrysantha (Hu) Tuyama.* on several in vitro activities, including anti-inflammatory and thrombolytic effects. Additionally, the study aimed to assess the acute toxicological activity of the extracts in an animal model.

**Place and Duration of Study:** The research study was carried out from Nov 2023 to Feb 2024 at the Bangladesh Council of Scientific and Industrial Research (BCSIR), Laboratory of Pharmacology, University of Asia Pacific and the Laboratory of Phytochemistry and Pharmacology, Department of Pharmacy, as well as the Laboratory of Microbiology at Stamford University Bangladesh, Dhaka.

**Methodology:** The methodology involved various doses of methanolic leaves extract of *Camellia Chrysantha (Hu) Tuyama*, and employed techniques such as the Inhibition of Protein Denaturation Assay for anti-inflammatory test and the clot lysis test for thrombolytic assay. The acute toxicological activity was assessed by Cinnamon oil induced toxicologicaland administration of acetic acid induced writhing responses in *Swiss albino* mice.

**Result:** The Inhibition of Protein Denaturation Assay test yielded findings indicating its anti-inflammatory properties against all strains used in this research. The findings from the study examining the anti-inflammatory properties indicate that *Chrysantha (Hu) Tuyama* exhibits a significant efficacy of 86.54% in inhibiting inflammatory at a concentration of 1000 µg/mL. This level of efficacy is comparable to that of acetylsalicylic acid, which also has a similar efficacy of 98.54 % at the same concentration. thrombolysis test is a controlled laboratory procedure that assesses the ability of plant extracts to dissolve blood clots. The study found that MECCL can reduce blood clots. Table 2 shows that MECCL's thrombolytic potential was 95.69%, which is much higher than the usual value of 91.304%. Our research found that MECCL did not induce any harmful effects in mice during the acute toxicity test, as determined by the toxicological assessment. No signs of illness or death were reported throughout the acute toxicity trial.

**Conclusion:**The *Camellia chrysantha (Hu)*Tuyama plant exhibits privileged qualities that could lead to the discovery of novel therapeutic substances. It has anti-inflammatory, thrombolytic, and mild acute toxicity properties. Further exploration of this plant could be beneficial. Our study results contribute to the growing body of evidence demonstrating that natural products may potentially have favourable effects on human health.

**Keyword:** anti-inflammatory, thrombolytic, toxicological activities, denaturation, clot lysis, Cinnamon oil induced.

## 1.INTRODUCTION

Traditional medicines utilize natural substances and are of vital value. Ayurveda, traditional Chinese medicine, traditional Korean medicine, and unani are all examples of medical practices that have been practiced all over the globe for hundreds or even thousands of years, and they have developed into well-organized medical systems. All of these practices make use of natural components. It is possible that they have been flawed in their various incarnations; yet, they continue to be an important source of human knowledge today(Shomudro et al., 2023).

Within the family Theaceae, the Camellia genus is the most significant in terms of size and commercial significance. An attractive plant planted all over the globe is called *Camellia chrysantha (Hu) Tuyama* (CCT). It is a member of a group of yellow-flowering species known as golden camellias, native to the southern region of China. People in South China often create tea out of CCT because of the wonderful benefits that it has from a medicinal standpoint(Wei et al., 2015).Research has shown that extracts derived from the leaves of CCT had antioxidant properties, as seen in experiments involving the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide anions, and hydroxyl free radicals. Abundant data indicates that oxygen-free radicals play significant roles in the amplification of inflammation(EL-Rahmany & Sharaf, 2023). CCT is known to possess numerous trace elements including germanium, selenium, manganese, molybdenum, vanadium, and zinc. The bioactive compounds present in this species can inhibit tumour growth by up to 33.8% and reduce blood cholesterol levels by up to 35%. The plant can alleviate the symptoms associated with atherosclerosis resulting from elevated levels of blood lipids. Furthermore, it may regulate blood pressure and serve as a treatment for cardiovascular ailments and diabetes(Bach et al., 2020).

In this study, the composition-activity relationship was used to identify the anti-inflammatory and toxicological components in CCT. The chemical profiles of various CCT preparations were characterized using ultra high-pressure liquid chromatography (UHPLC) coupled with linear ion trap hybrid orbitrap MS, which could perform a rapid separation while utilizing the efficacy of UHPLC and providing accurate structural information about the constituents via the high-resolution of orbitrap MS (Magalha˜es, et al., 2008). Meanwhile, to evaluate the scavenging rate of free radicals, a DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging test that is stable, commercially available, and does not require prior generation was used. This test is used in about 90% of anti-inflammatory investigations. The association between chemical profiles and antioxidant activity was discovered to be significantly(Moon et al., 2009).

The primary objective of the investigation was to ascertain the anti-inflammatory, thrombolytic, and acute toxicological actions of bioactive metabolites derived from the leaves of *Camellia chrysantha* in vitro and in vivo, respectively.

## 2. MATERIAL AND METHODS

### 2.1 Plant material

The Camellia chrysantha specimen was obtained in October 2023 from its location at 1217 Moulana Bhasani Road, Romna Park, Dhaka, Bangladesh. The plant (DACB 88045) was definitively identified by a group of experts from the Bangladesh National Herbarium in Mirpur, Dhaka. After being acquired, the plant was allowed to desiccate in a shaded area for eleven days. Subsequently, it was pulverized into a fine particulate form to be used in experimental procedures.

### 2.2 Preparation of plant extract

The *Camellia chrysantha* was taken as a whole and pristine plant after the removal of soil. Subsequently, the whole plant was washed with water at ambient temperature to eliminate any residual dust. After cleaning, the *Camellia chrysantha* was left to undergo natural drying in a shady location for a period of twelve to fifteen days. Before being mechanically pulverized into minute fragments and blended, the plant underwent air-drying. During three days, a quantity of 58 grams of *Camellia chrysantha* powder was fully submerged in a volume of methanol equivalent to three finger widths.Following drying, the leaves were pulverised with an electric blender. The solid material was then blended with 1200 mL of methanol in a hermetically sealed container for 10 days, with regular agitation. The mixture was filtered using cheesecloth, and the resulting filtrate was further filtered through Whatman filter paper. The filtrate was concentrated using a water bath and then dried in an oven at 40°C. The resultant extract was then transferred to an airtight container and stored in a refrigerator(Ayshi et al., 2023).Periodically, the act of stirring facilitated the maceration process. The extract underwent filtration using filter paper after three days. After the solvent was allowed to evaporate, a total of 5.69 grams of extract was obtained. The unprocessed extract was stored in a beaker, ensuring it remained undisturbed and shielded from light(Shaira et al., 2023).

### 2.3 In-vitro anti-inflammatory activity

The previous study reported using this approach with minimal modifications(Chowdhury et al., 2023). To create the reaction mixture (5 mL), 0.2 mL of egg albumin (derived from a fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of MECC were combined at concentrations of 31, 62.5, 125, 250, 500, and 1000 µg/mL. Comparative controls included the same amount of water that had been double-distilled. Afterwards, a BOD incubator (Labline Technologies) was used to incubate the solutions

at 37°C for 15 minutes after heating them to 70°C for 5 minutes. After they had cooled, we measured their viscosity using an Ostwald viscometer and their absorbance at 660 nm (SHIMADZU, UV 1800) with a vehicle blank. Diclofenac sodium was used as a reference medicine at final concentrations of 31, 62.5, 125, 250, 500, and 1000 µg/mL while the viscosity and absorbance were determined. The following formula was used to ascertain the percentage of protein denaturation that was averted:

$$\% \text{ of Inhibition} = \frac{Abs\ of\ control - Abs\ of\ test\ sample}{Abs\ of\ control} \text{ X } 100$$

Here, Abs= Absorbance

**2.4Thrombolytic assay**

**2.4.1 Blood sample**

15 healthy human volunteers who had never used blood-thinning medication, nicotine, or oral contraceptives had their venous blood collected (with the help of a medical expert). Stamford University Bangladesh's Institutional Ethics Council gave institutional ethical approval to the whole procedure. 15 microcentrifuge tubes were then filled with 500 µL of fresh blood.

**2.4.2 Affirmation of donors' consent**

Every single donor was supplied with a consent form that narrated the purpose of this research, title of this project, and the volume of blood that will be drawn. The illustration of this research includes whether or not volunteers will consume any therapy, any kind of irritation to the piercing area and the time period for blood collection.

**2.4.3 Clot lysis method**

Using pre-weighed sterile vials holding 1 mL each, blood was taken from healthy participants and then divided into vials of 5 mL. At 37°C, blood samples needed 45 minutes to coagulate. A fresh weight measurement was made to determine the clot weight after the generated serum was emptied from the vials. Next, a 100 µL water solution containing the MECCL (2 mg/mL) was added to the vials. One hundred microliters of a non-thrombolytic control and thirty thousand units of streptokinase were used as standards. The next step was to transfer the mixture to an incubator set at 37°C after 90 minutes. Measurement of the changed weights of the vials after incubation and drained the fluid that the clot had produced was noted (Bhuiyan et al., 2023). To measure the thrombolytic activity, the following equation was employed to calculate the percentage of clot lysis.

$$\% \text{ of Clot lysis} = \frac{A}{B} \times 100$$

where A and B reflect, respectively, the weight of the released clot before treatment and after treatment.

**2.5 In-vivo acute toxicological test**

### 2.5.1 Experimental animals

We used young, healthy Swiss albino mice weighing between 19 - 26 g apiece as our experimental participants. We obtained our mice from ICCDRB, located in Mahakhali, Dhaka, Bangladesh. Ensuring a fundamental state was of utmost importance. Typical environmental parameters include a temperature and humidity of 25 °C (77°F) with a relative humidity ranging from 55-65%. Additionally, a 24-hour light/12-hour dark cycle is also considered standard. The aforementioned conditions are maintained constant for 8 days after collection. Following ICCDRB's requirements, we administered mice with a suitable meal and filtered water to aid their recuperation from the lack of water and food they experienced during transportation, and to assist them adapt to the laboratory environment before their use in any tests. The mice were prepared for the experiment after 5 days of rest.

### 2.5.2 Cinnamon oil induced toxicological test

Each group consisted of 5 mice that were administered either 1000 mg/kg, 2000 mg/kg, or 3000 mg/kg of MECCL and Cinnamon oil orally. The control group received water as a vehicle. Following 24 hours of observation, mortality rates were recorded for both cohorts(Hossein Hosseinzadeh, et al.,2002).

## 3. STASTICAL ANALYSIS

The bioassay measurements were performed three times, and the tabular data is presented as the average standard deviation. Excel was used for doing statistical analysis.

## 4. RESULTS AND DISCUSSIONS

### 4.1 Anti-inflammatory activity

Both MECCL had a more noticeable impact than standard, acetylsalicylic acid, according to the results shown in Table 1.

**Table 1.** Protein denaturation (egg albumin) assay results

| Samples | Concentrations (ug/ml) | % of inhibition |
|---|---|---|
| Acetyl salicylic acid | 62.5 | 91.82 |
| | 125 | 93.91 |
| | 250 | 94.78 |
| | 500 | 96.72 |
| | 1000 | 98.54 |
| MECCL | 62.5 | 62.37 |
| | 125 | 75.55 |
| | 250 | 81.33 |
| | 500 | 83.64 |
| | 1000 | 86.54 |

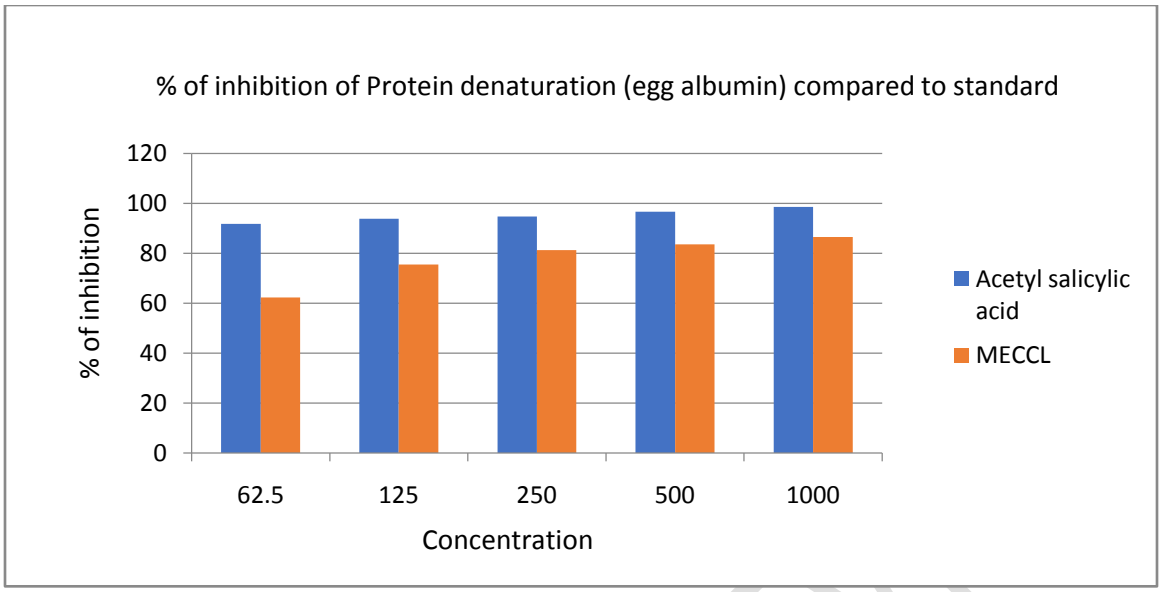


**Figure 1.Percentage of inhibition of Protein denaturation (egg albumin) compared to standard**

The anti-denaturation technique of egg albumin was selected for the assessment of MECCL's anti-inflammatory function. The anti-denaturation approach involves denatured egg albumin in the experiment. The denaturation process is brought about by applying heat. Proteins that have been denatured express certain antigens (Adetutu & Olukorede, 2021). These antigens are linked to type-III hypersensitivity responses, which in turn are linked to certain illnesses including serum sickness and glomerulonephritis. Not only that but it has previously been shown that traditional NSAIDs such as indomethacin and phenylbutazone do more than only block the COX enzyme, which is responsible for the endogenous prostaglandin's synthesis. Plus, they prevent proteins from becoming denaturized(Adetutu & Olukorede et al., 2021), (Shomudro et al., 2023).

That is why the anti-denaturation test is the most practical way to screen for anti-inflammatory efficacy. The results of this investigation demonstrate that the extract has potent anti-inflammatory properties. Autoantigen synthesis may be regulated by *Camellia chrysantha (Hu) Tuyama*. Protein denaturation may therefore be prevented. A standard medication was used to compare this effect. The comparison was based on aspirin, the typical medicine. Preliminary phytochemical screening revealed the presence of secondary metabolites such as alkaloids and flavonoids(Conforti et al., 2008), (Yesmin et al., 2020).

Medicinal plants are the main source of medications. Because of their cost, accessibility, and cultural acceptance, they are approved medicines independently and/or in combination with synthetic treatments in both developed and developing nations for the treatment of disorders such as inflammatory diseases (Mutuma et al., 2020). According to research reports, 120 plant-derived chemicals serve a purpose in

Western medicine, and around 80% of the world's population relies on medicinal plants for primary health care. Phytochemicals derived from medicinal plants are effective in treating a variety of inflammatory illnesses (Ayertey et al., 2020)

### 4.2Thrombolytic activity

An in vitro thrombolysis test is a controlled laboratory procedure that assesses the ability of plant extracts to dissolve blood clots. To prevent more bleeding after an injury, proteins and blood cells clump together to form a clot. On the other hand, conditions like heart attacks and strokes may be deadly if clotting occurs too often. Comparing MECCL to the standard streptokinase, the following data shows that MECCL has a much higher percentage of clot lysis.

**Table 2. Thrombolytic activity (in terms of % of clot lysis) of the extractives of MECCL**

| Sample | % of clot lysis |
|---|---|
| Negative control | 7.296 |
| Streptokinase | 92.467 |
| MECCL | 94.59 |

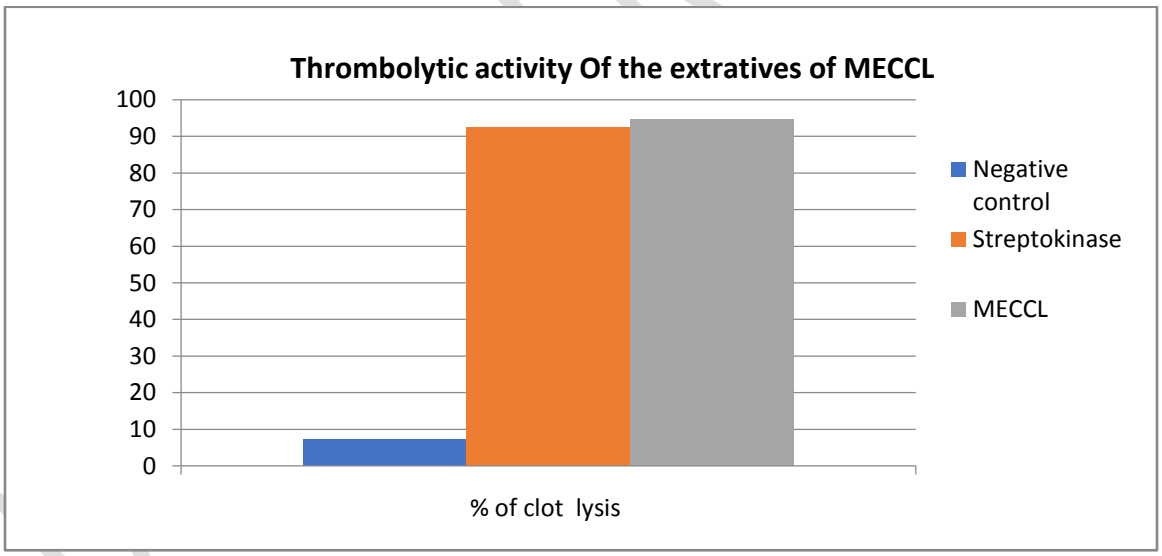


**Figure 2. Thrombolytic activity (in terms of % of clot lysis) of the extractives of MECCL**

The methanol extract of *Camellia chrysantha (Hu) Tuyama* (leaf) showed thrombolysis of 95.69% respectively, in this study. Since this is just a preliminary examination, the chemical and pharmacological properties of the extract should be further investigated to maximise their medicinal and pharmaceutical potential.

A wide range of research investigations have sought to identify natural food sources, supplements, and botanicals with thrombolytic action for the treatment of coronary

events and strokes. The study found that MECCL can reduce blood clots. Table 2 shows that MECCL's thrombolytic potential was 95.69%, which is much higher than the usual value of 91.304%. This result was achieved because MECCL reduces blood clotting in laboratory studies, leading to the notion that it has cardioprotective properties (KUNWAR et al., 2022). The MECCL has great potential for improving cardiovascular health and might pave the way for the development of new thrombolytic medicines derived from the leaf of the *Camellia chrysantha (Hu)* plant (Uddin et al., 2021).

### 4.3 Acute toxicological activities

In the acute toxicity test, the methanolic extract was administered at doses of 1000, 2000, and 3000 mg/kg, while cinnamon oil was administered at a dose of 20 mg/kg. Both substances caused a delay in the onset of seizures compared to the negative control group. The mortality protection rate for convulsion survivors was 5 out of 5 animals tested in the 1000 mg/kg methanolic extract group, 5 out of 5 animals tested in the 2000 mg/kg methanolic extract group, and 4 out of 5 animals tested in the 3000 mg/kg methanolic extract group. These rates indicate that the methanolic extracts were less effective compared to Cinnamon oil, which showed no mortality protection at a dosage of 20 mg/kg.

**Table-3: Effect of MECCLon mice.**

| Treatment | Onset time of seizure (s) | Mortality protection after 30min | Mortality protection after 24h |
|---|---|---|---|
| Normal saline | 27±2.95 | 0/5 | 0/5 |
| Cinnamon Oil (20mg/kg) | 343±3.76 | 5/5 | 5/5 |
| MECCL (1000mg/kg) | 20±2.21 | 0/5 | 0/5 |
| MECCL (2000mg/kg) | 85±3.09 | 0/5 | 0/5 |

| | | | |
|---|---|---|---|
| **MECCL (3000mg/kg)** | **116±2.72** | **0/5** | **1/5** |

The main natural compounds present in yellow Camellia include α-spinasteryl-D-glucopyranoside, stigmata glucopyranosides, aromadendrin, catechin, phlorizin glucopyranosides, dodecanoic acid, 3β-acetoxy-20-lupanol, and 3β,6α,13β-trihydroxyolean-7-one. The water extract derived from yellow Camellia has shown anti-anxiety, antidepressant, and anti-bacterial properties(Nguyen et al., 2020). Our research found that MECCL did not induce any harmful effects in mice during the acute toxicity test, as determined by the toxicological assessment. No signs of illness or death were reported throughout the acute toxicity trial. The $LD_{50}$ value of the extract was not determined, however, it probably exceeds 3000 mg/kg, indicating that the extract is essentially harmless and suitable for oral administration at the tested doses. Notably, no notable disparities in food and water consumption, weight increase, or biochemical or hematological factors were found between the control and treatment groups during oral administration.

## 5. CONCLUSION

The *Camellia chrysantha (Hu) Tuyama* plant, belonging to the family Theaceae, has shown potential health benefits in in vitro and in vivo studies. This plant has auspicious characteristics that might potentially result in the development of innovative medicinal compounds. It has anti-inflammatory, thrombolytic, and modest acute toxicological action. Further investigation of this plant might be beneficial. According to the study, it is crucial to investigate the possible health advantages of traditional medicinal herbs, as they may serve as significant bases for the creation of novel medications. Further in vivo and clinical investigations are required to ascertain the effectiveness and safety of this plant for human consumption. Overall, our study results add to the increasing body of evidence indicating that natural products might potentially have beneficial impacts on human health. This highlights the need for further investigation into the therapeutic potential of plants used in medicine industries.

## 8. ETHICAL APPROVAL

This research followed all rules set forth by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonization. Stamford University Bangladesh's Faculty of Science examined and accepted the research procedure and written consent form (reference number: SUB/ERC/202309). Everyone

who took part in the study had to submit a documented consent form, and they had the right to withdraw at any moment.

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