Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(3), 1208-1225 | ISSN:2251-6727

Evaluation of Anti-Thrombotic Activity of Clerodendrum Colebrookianum Walp in Albino Wistar Rats

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(Received: 04 August 2023 Revised: 12 September Accepted: 06 October)

KEYWORDS
Clerodendrum Colebrookianum, hyperactivity, platelet activity, coagulation, antithrombotic actions

ABSTRACT:
It has been widely held that Clerodendrum colebrookianum Walp was used by ancient herbalists to treat blood stagnation and inflammation. Several physiological functions have been associated with the leaves of Clerodendrum colebrookianum Walp. But their antiplatelet and antithrombotic effects are a complete mystery. Therefore, the purpose of this study is to evaluate the effects of chloroform and hydroalcoholic extracts (CBC & CBH) of Clerodendrum colebrookianum Walp leaves on platelet activity, coagulation, and thromboembolism. In an in vivo study of carrageenan-induced rat tail thrombosis, C.B.C.E. and C.B.H.E. significantly reduced thrombosis. C.B.C.E. & C.H.E. also reduced swelling and redness in the tails of rats. The antithrombotic action of the thrombosis management and its consequences in the present experimental investigation may be accounted for by the presence of flavonoids, phenolic compounds, alkaloids, glycosides, and sterols in the extract of chloroform and hydro-alcoholic (40%) solvents. Drabkin's tail bleeding experiment in rats also showed that C.B.C.E and C.B.H.E given orally provided protection. Contrasting the standard treated groups and the normal groups, the cyanmethhemoglobin method's estimates of haemoglobin level at different time intervals raises encouraging thoughts of superior anti-platelet and anticoagulant activity. Flavonoids, phenolic compounds, alkaloids, glycosides, and sterols may be responsible for the antithrombotic actions of chloroform and hydro-alcoholic extracts of C.B.C.E and C.H.E. This research suggests that CBC and CBH have therapeutic and preventive potential for cardiovascular illnesses associated with platelet hyperactivity.

1 Introduction:

After arterial wall damage, a blood clot controls bleeding. When a blood vessel wall breaks, hemostasis must be fast and effective. Steady, focused, and controlled reply is needed. Broken blood clotting and hemostasis systems may cause thrombosis and irregular bleeding. Damaged platelets activate the sub-endothelial matrix receptor and agglomerate. A blood clot blocking blood flow causes thrombosis [1-2].

Many disorders are linked to arterial and limb ischemia. Myocardial infarction, stroke, and PE are examples. 80% of deaths in underdeveloped nations are from cardiovascular illness (heart attack or stroke). Thrombuses occur when platelets aggregate during arterial wall damage. ADP, fibrinogen, calcium, serotonin, PDGF, and thromboxane A2 stimulate platelets. Anticoagulants, antiplatelets, or fibrinolytics treat arterial and venous thrombosis [3-7].

Aspirin and clopidogrel are used instead of heparin and warfarin [8-11].

TPA, streptokinase, and reteplase treat blood clots and thrombosis. If you're concerned about major side effects including bleeding, allergic reactions, thrombocytopenia, stomach ulcers, cardiovascular events, or therapeutic resistance, these medications may not be good for you Natural commodities are preferred because they are risk-free and may be substituted. In 2003, the WHO found that developing nations treat common ailments using traditional medicinal herbs. Ethno-medicinal plants provide healthcare for 80% of the world's population [12-19].

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www.jchr.org
JCHR (2023) 13(3), 1208-1225 | ISSN:2251-6727

Northern India is culturally and biologically diverse. North Carolina's tropical climate supports 500 traditional medicinal herbs. These herbs have been used by several indigenous North American societies for healing [20-23].

Ethnobotanists praise the Clerodendrum colebrookianum Walp plant, which is utilised by the Apatani, Mishing, Nishi, Mizos, Jantias, and others. Pakkom, Nefafu, and Phuinum are its common names. Proponents believe it may treat hypertension, diabetes, hypercholesterolemia, and parasites. Local groups have their own meals, drinks, and ceremonies. Assamese Mishing drink Clerodendrum colebrookianumin Apong, a rice beer. On New Year's Day in Manipur Sajibucheiroba, "uti" a natural carbonated mixed vegetable porridge is made using Clerodendrum colebrookianum to keep the community well [24-28].

2 Materials and Methods:

2.1 Plant Collection and Identification

In the month of October, we gathered Clerodendrum colebrookianum Walp leaves from a local market in Bijnor, Uttar Pradesh and identify by Dr. V Rama Rao Research Officer Botany Department of Central Ayurveda Research Institute Uttarahalli Clerodendrum colebrookianum Walp leaves were air dried at 37 to 40 degrees and ground to a coarse powder using a mesh size of 20. The leaves of Clerodendrum colebrookianum Walp were ground into a fine powder and extracted using the Soxhlet method and a succession of solvents that decreased in elutriation potential.

2.2 Research Chemicals:

Analytical-grade chemicals and reagents were utilised in all experiments. The extraction process included the use of chloroform and ethanol. The gold standard medication was sodium heparin. Drabkin's reagent was used to measure hemoglobin levels in a tail model assay for Cyanmethaemoglobin, and other reagents included nitro blue tetrazolium (NBT), 2-2-di phenyl-1- picryl hydrazyl (DPPH), gallic acid, ascorbic acid, sodium carbonate, the Folin-Ciocalteau reagent, ethylenediaminetetra acetic acid (Table.1).

Table 1: List of chemicals

SNo.	List of chemicals used
1	Nitro blue tetrazolium (NBT), 2-2-diphenyl-1-picrylhydrazyl (DPPH)
2	ccccccvcAscorbic acid
3	Gallic acid
4	Sodium carbonate
5	Folin-Ciocalteau reagent
6	Ethylene-di-amine-tetra-acetic-acid (EDTA)
7	Hydrochloric acid (HCL)
8	Carrageenan Powder (for in-vivo procedure)
9	Heparin sodium injection and Normal saline 0.9%
10	Drabkin’s reagent

2.3 Experimental Animals:

The research relied on Wistar rats (weighing between 150 and 250g). The Kumaun University, Bhimtal Campus, Nainital Uttrakhand, Provided the animals used in this study. All of the animals were housed in stainless steel-topped plastic rat cages. The animals benefited from a controlled habitat with an ideal photo period (12hours of darkness followed by 12 hours of light) and temperature (252). Normal food and running water were provided for the animals. The IAEC gave its stamp of approval to the research after reviewing it against the Purpose of Control and Supervision on Experimental Animals standards.

2.3.1 Methods:

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Step.1: Getting the Extract Ready:

For 14 days, Clerodendrum colebrookianum walp leaves (400g) were dried in the shade after being sorted, cleaned, and weighed. A powder was made by grinding the dried leaves. Using a soxhlet device, the fine powder of leaves was extracted with solvents of increasing polarity, such as chloroform and hydro alcohol (40%) in consecutive order. Plant material extracts were filtered and vaccum dried before being dissolved in water or solvents for in-vitro and in-vivo testing.

2.3.1.1 Phytochemical Analysis:

In order to determine the chemical components (such as phenols, tannins, glycosides, alkaloids, carbohydrates, saponins, terpenoids, flavonoids, and amino acids, fats, and fixed oils) present in plant material extracts (chloroform and hydro-alcohol), a Phytochemical analysis was performed. We modified the procedures described by Trease and Evans, Harborne to conduct our phytochemical analysis [29-30].

2.3.1.2 Phenolic Compound Testing:

The extract was dissolved in about 50mg in 5ml of distilled water for the phenolic compound assay. Also, few drops of ferric chloride solution (5% concentration) were added subsequently. When a dark green tint develops, phenolic chemicals are present [31].

2.3.1.3 Tannin Analysis:

A few millilitres of extract diluted in pure water. Then, include a 10% NaCl and 1% Gelatin solution. The presence of tannins may be verified by the appearance of white precipitation [32].

2.3.1.4 Flavonoid Analysis:

In a water bath, heat 10 ml of ethyl acetate and the plant extract for 3 minutes. Then, sieve the concoction. Add 1millilitre of the diluted ammonia solution to 4millilitres of the filtered mixture and shake. The presence of flavonoids is indicated by the emergence of a yellow tint [33].

2.3.1.5 Alkaloids Test:

a) Dilute HCl will be added to 50mg of solvent-free extract, which will then be mixed well before being filtered. Wagner's reagent will be applied to the filtered section. The presence of reddish-brown precipitates suggests the extract contains alkaloids. The plant extract was diluted to 1ml and subjected to Dragendroff's reagent. The presence of alkaloids is indicated by an orange-red colour change in the presence of the reagent [34].

2.3.1.6 Steroid Testing:

Add a few drops of Liebermann Burchard Reagent to 1 millilitre of the extract. The presence of steroids is indicated by the mixture taking on a greenish hue [35].

Hesse's Test (b):

Dissolve 50mg of the extract in 4ml of chloroform in a test tube; gently add concentrated H2SO4 next to the test tube. Steroids are represented by pink since they are toxic to humans.

2.3.1.7 Examining for Saponins:

Two grammes of the plant powder was added to ten millilitres of water in a test tube and shaken vigorously. The presence of saponins may be confirmed by hydrolysis with 2N HCL decreased Fehlings' solution, which should provide a soapy liquid [36].

2.3.1.8 Carbohydrate Analysis:

Fifty milligrammes of the extract were dissolved in sterile water and filtered. The filtered solution was then combined with one millilitre of Fehling's solution and brought to a boil. The presence of carbohydrates was verified by the formation of red precipates [37].

2.3.1.9 Glycosides Testing:

The Keller-Killiani Index: After adding a little amount of glacial acetic acid to the extracts (1 ml), we allowed the mixture to cool and then added a few drops of ferric chloride. These two components were then added to 1 ml of concentrated sulphoric acid in a separate test tube. The presence of glycosides was noted by the

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transformation of the reddish brown layer to a bluish-green hue [38].

2.3.1.10 Terpenoids Analysis:

As for the Salkawski, in this test, a few milliliters of the extract were combined with two millilitres of chloroform, and then three millilitres of strong sulphoric acid was introduced to the test tube's sites very gradually. The presence of terpenoids may be confirmed by the formation of a reddish-brown ring in the content of a test tube [39].

2.3.1.11 Fixed Oil and Fat Analysis:

A little bit of extract was spread in between the two filter sheets and squeezed. The presence of a greasy smear on the filter paper suggested the presence of fixed oils [40].

2.4 Antioxidant Activity Test, In Vitro:

2.4.1 Phenolic Content Prediction:

The Folin-Ciocalteau reagent and technique may be used to determine the total phenolic content. This method is based on the oxidation activity or reaction. In this experiment, gallic acid served as a standard. In order to reduce the concentration of the extract solution to 1.0 g/ml, 46 ml of distilled water was added to the flask. The extract was diluted with 1 ml of the Folin-Ciocalteau Reagent before addition, and then that was added to the mixture and stirred well. The solution was left to rest for three minutes after the components had been well mixed. After mixing in the sodium carbonate, we let the mixture to rest for 180 minutes, shaking it occasionally. At 760nm, we saw the blue cast. The gallic acid content of several phenolic compounds was quantified. (GAE) [41].

2.4.2 Hydrogen peroxide (H2O2) scavenging activity assay:

The hydrogen peroxide scavenging test was established, based on Ruchet-technique with certain modifications. The sample (1ml; 10-320 g/ml) was incubated at room temperature for 10 minutes after being mixed with PBS (2.4ml; 0.1M) and hydrogen peroxide solution (0.6l; 40%). At 230nm, the absorbance of the reaction was determined. Ascorbic acid was employed as a positive control [42].

The results of the hydrogen peroxide scavenging test are shown below:

$$\% \text{ Inhibition} = \left(\frac{A1-A2}{A0}\right) \times 100$$

2.4.3 Reducing Power Assay:

Oyaizu (1994) evaluated an assay for power reduction in this way. Extracts were mixed with sodium phosphate buffer (pH 6.6; 2.5 ml; 200mM) and potassium ferric cyanide (pH 6.6; 2.5 ml; 1%) in quantities ranging from 125 to 800millilitres per milliliter. After 20 minutes of mixing at 50°C, 2.5 ml of 10% w/v tri-chloro-acetic acid was added to the mixture. After the solution was well mixed by swirling and mixing, it was centrifuged for 8 minutes at 1000 rpm to separate the layers. The top layer was estimated after centrifugation. To five millilitres of deionized water, one millilitre of ferric chloride at 0.1% and five millilitres of the top layer were added. After proper mixing, the absorbance at 700 nm was determined using a twin beam spectrophotometer. After repeating the process three times, we had enough data to calculate means and variances. The EC50 value was calculated using absorbance/concentration data and the analytical standard ascorbic acid.

2.5 Determination of DPPH radical Scavenging Assay:

DPPH (2, 2-diphenyl-2-picrylhydrazyl) free radical was used to determine the antioxidant properties of any chemical by evaluating the DPPH free radical's activity. We generated an ethanol-based DPPH solution (0.1mM) that we then added to 3ml of extract solution made at various doses (1-5g/ml) and mixed well. After 30 minutes of dark incubation, the absorbance at 517nm was measured. The reference chemical was ascorbic acid. This method's outcome was computed using the formula below and given as a percentage of DPPH scavenging effect [43]:

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$$\% \text{ Inhibition} = \frac{Ao - At}{Ao} \times 100$$

Where,

A_0 = absorbance of blank.

A_t =absorbance in presence of extract.

2.6 Estimation of Superoxide radical-Scavenging Activity:

Nitro blue Tetrazolium (NBT) was used by Sabu and Ramadasan to measure scavenging activity (2002). In a test tube, extracts of various concentrations (12.5-400g/ml) were collected. Sodium carbonate (50mM), NBT (24mM), and EDTA (0.1mM) solutions were poured into a test tube and the absorbance at 560 nm was immediately measured. The reaction was started with the addition of 0.4ml of hydroxylamine hydrochloride (1 Mm). At 560 nm, NBT was reduced in the reaction mixture for 15 minutes at 25 degrees Celsius. Absorption of the reaction mixture decreased as a result of an increase in superoxide anion activity. The standard compound was ascorbic acid [44].

All the extracts were treated similarly and percentage inhibition was calculated as follow:

$$\% \text{ Inhibition} = \frac{Ao - At}{Ao} \times 100$$

Where,

A_0 = abs. of blank.

A_t = abs. in presence of extract.

2.7 In-Vivo assessment of Anti-platelet Activity by Using Plant Extract:

2.7.1 Carrageenan Induced Rat tail thrombosis assay:

Carrageenan-induced rat tail thrombosis model was used to test the anti-thrombotic efficacy of Clerodendrum colebrookianum Walp leaf extracts. Many different physical and chemical methods (98) have been utilised to stimulate the formation of thrombi in animals used in scientific experiments. Carrageenan, a muco-polysaccharide isolated from the walls of red algae, has, nonetheless, been the chemical of choice because of its useful properties. There is no requirement for the killing of animals, therefore it may be utilised for in-vivo studies, and the decreased number of animals required is ethically sound [45].

In addition, extensive surgical procedures to expose the blood vessels in the animals' tails are unnecessary. Carrageenan may potentially influence the inactivation of Hageman factor, which results in endogenous coagulation. The carrageenan-induced thrombus model assay may be used to evaluate the efficacy of thrombolytic drugs such heparin, urokinase, streptokinase, and aspirin. Therefore, we adopted a carrageenan-induced tail model experiment in the present study to assess the antithrombotic activity of the plant Clerodendrum colebrookianum Walp. Wistar rats, which usually weigh between 200 and 250 grammes, were used in the experiment. The animal enclosures were maintained at a constant temperature of 22.3 degrees Fahrenheit and a light/dark cycle of 12 hours. All animals were given the same treatments (IAEC) and allowed to drink tap water and eat pellets as usual for at least two weeks prior to the experiments, during which time the antiplatelet activity of a Clerodendrum colebrookianum Walp extract was investigated in a rat model of carrageenan-induced tail thrombosis. There were nine groups, each including six animals, and all of them had tails longer than 13 cm. Carrageenan was dissolved in saline and injected into the dorsal vein of each animal's tail at a dose of 2 mg/kg. Clerodendrum colebrookianum Walp extract was tested for its antithrombotic efficacy in a carrageenan-induced rat tail thrombosis model. Animals with tails longer than 13 centimetres were split into nine groups of six. All animals received a 2 mg/kg injection of carrageenan dissolved in saline into the dorsal tail vein [46].

Animal grouping:

Group-I: Saline (2 ml/kg)
Group-II: Carrageenan (2 mg/kg)
Group-III: Carrageenan (2 mg/kg) + Heparin (100 IU)
Group-IV: Carrageenan (2 mg/kg) + Clerodendrum colebrookianum Walp choroform extract (100 mg/kg)
Group-V: Carrageenan (2 mg/kg) + Clerodendrum colebrookianum Walp chloroform extract (200 mg/kg)

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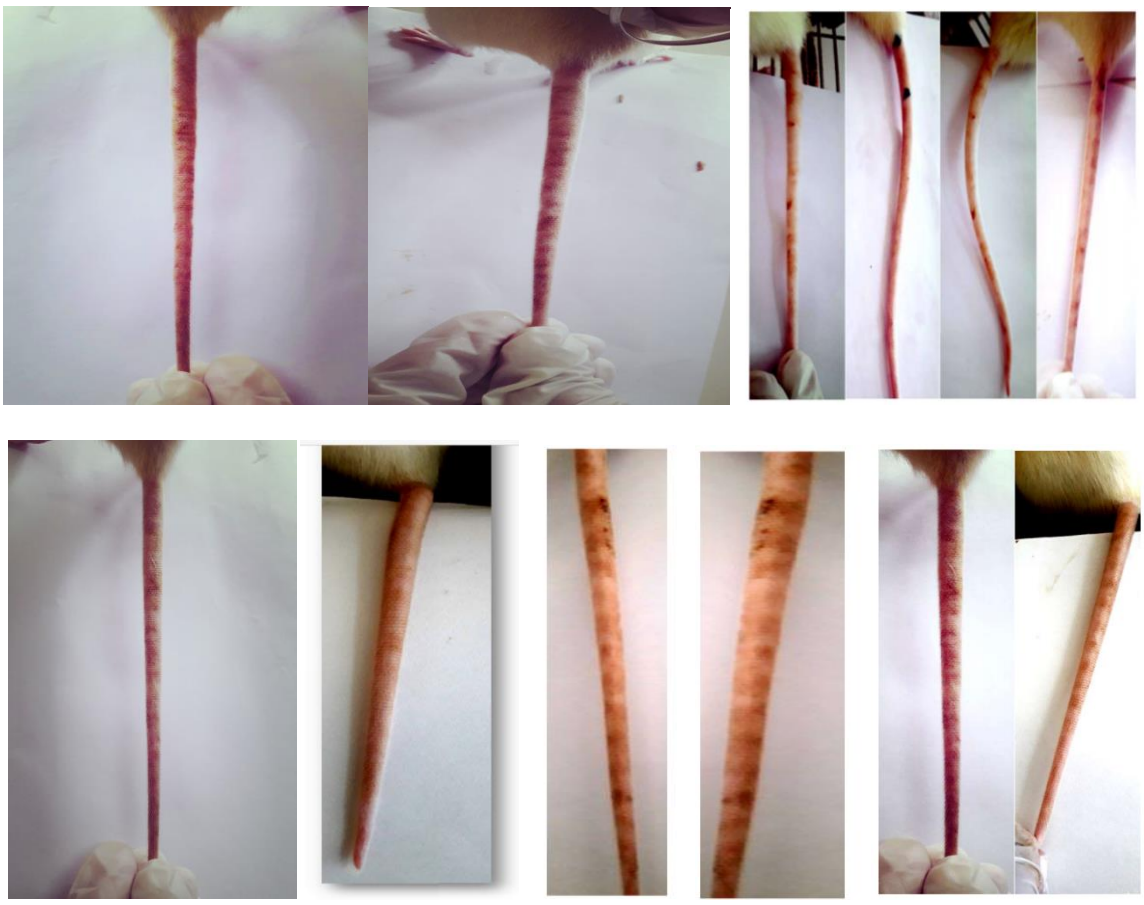
Group-VI: Carrageenan (2mg/kg) + Clerodendrum colebrookianum Walp chloroform extract (400 mg/kg)
Group-VII: Carrageenan (2mg/kg) + Clerodendrum colebrookianum Walp hydro-alcoholic extract (100mg/kg)
Group-VIII: Carrageenan (2mg/kg) + Clerodendrum colebrookianum Walp hydro-alcoholic extract (200mg/kg)
Group-IX: Carrageenan (2mg/kg) + Clerodendrum colebrookianum Walp hydro-alcoholic extract (400mg/kg)

After waiting 10 minutes, Group III received 300 iu/kg of heparin, whereas Groups IV, V, VI, VII, VIII, and IX received 100, 200, and 400 mg/kg of Clerodendrum colebrookianum Walp extract, respectively. Six, twenty-four, and forty-eight hours after the carrageenan injection, the incidence of infarction and the length of the infarcted area of the tail tip were recorded. The placebo group received merely saline solution.

2.7.2 Evaluation of thrombosis and antithrombotic activity:

2.7.2.1 Thrombosis of tail:

The redness and swelling of rat tails were monitored for a period of six hours, twelve hours, 24 hours, 48 hours, and 72 hours. Below are some examples of my work.



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2.7.2.2 Bleeding Time:

The animal was restrained in a crate. One of the opening's ends was used to pass the tail through. After a thorough examination of the animal's tail, a massage was given to where it meets the pelvis. Spirulina had been applied to the tail before it was dried and punctured approximately 4-mm deep. At 30-second intervals, a filter paper was used to clean the flowing blood. The procedure was repeated every 30 seconds on a new filter paper place until the bleeding had ceased. There was a stop-watch used to keep time.

2.7.2.3 Clotting Time:

The animal was restrained in a crate. One of the opening's ends was used to pass the tail through. After a thorough examination of the animal's tail, a massage was given to where it meets the pelvis. Spirulina had been applied to the tail before it was dried and punctured approximately 4-mm deep. The centre of the slide has a blood drop on it for testing purposes. With the use of a pin, a little blood droplet was slowly raised 30 second intervals. When coagulation was seen, the procedure was preceded. In seconds, the coagulation time was recorded.

2.7.2.4 Statistical analysis:

The mean standard error of the mean (n = 6) was used to summarise the study's findings. After doing a one-way ANOVA, we used Tukey's test to identify significant differences between groups. The p 0.05 and p 0.01 levels of statistical significance were used.

2.7.2.5 Drabkin's Tail bleeding Assay:

In a tail-bleeding experiment using Wistar rat tail tip amputation, this is the easy, low-cost, and more sensitive approach for determining bleeding patterns and haemoglobin content. The cyanmethhemoglobin test is one of the most reliable procedures for estimating heamoglobin. After severing the tail, the Hb concentration was evaluated in this study. After an hour of fasting and weighing, the animals were given the compounds orally. After injecting the animals for an hour, we gave them 100mg/kg of ketamine to put them to sleep. The blood was collected using 5cc of Drabkin's reagent after severing the tail's distal 5 cm vertically at a 90° angle (Fig.12). This chemical causes an irreversible reaction between lysed haemoglobin & potassium cyanide or potassium ferricyanide. Cyanomethaemoglobin, a stable blue pigment, is formed when haemoglobin (Hb) is oxidised. After 30 minutes of gentle homogenization, the 500 litres were divided among five test tubes (100litres each) for the bleeding tails and Drabkin's solution. After each transfer, 1.5cc of reagent was added to the Drabkin. Spectrophotometric analysis of absorbance at 540nm was used to determine haemoglobin concentration [47]:

Hb amount (g/dl) equals sample absorbance multiplied by the calibration factor.

2.7.2.6 Drabkin's method (Cyanmethaemoglobin method):

It is also known as hemoglobin cyanide method and this method is one of the most accurate methods for hemoglobin determination.

Procedure:

The procedure begins by transferring reagent 5ml in to two test tubes one for the standard and one for the test subject. Then, add 20µL of specimen (blood sample) in to the second test tube containing 5ml of reagent. Rinse pipette 3-5 times and mix well in the test tube and stand for 10mins to allow full conversion.

Drabkin's reagent + Blood
Potassium ferricyanide $\longrightarrow$ Hemoglobin Methemoglobin (oxidized hemoglobin) also called Hemiglobin.

Then, potassium cyanide binds to Methemoglobin and makes cyanmethemoglobin also called (Hemiglobin cyanide). After 10mins the blank test tube is read at 540nm and the absorbance is adjusted to zero or 100% transmittance. Later, read the specimen tube with the blood sample and record the absorbance reading in the spectrophotometer screen and compute for the Hb.
We use the following formula to calculate the Hb level.

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Conc. of unknown/specimen

$$= \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \text{x conc. of standard (g/dl)}$$

3 Results and Discussion:

3.1 Extraction:

Size reduced powder of Clerodendrum colebrookianum Walp leaves were extracted successively, by soxhlet extraction technique with solvents down the elutropic series, viz. chloroform & hydro-alcohol (40%) at (40-60 °C). The extractive yield of plant from respective solvents is mentioned in (Table.2).

Table.2: Extraction yield of plant

S/No	Extract	%w/w yield of the Clerodendrum colebrookianum Walp leaves
1	Chloroform	3.76
2	Hydro-alcoholic (40%)	14.06

3.1.1 Qualitative Phytochemical screening:

The qualitative analysis of extracts from plant material showed the presence of various phytochemical constituents (Table.3).

Table.3: Qualitative analysis of plant

Phytochemical	Name of test	Indication	Outcome	
			Chloroform	Hydro – alcohol
Flavonoids	Ferric chloride	Light of dark green color	Absent	Present
	Alkaline reagent	Intense yellow color; disappears on addition of dilute acid	Absent	Present
Alkaloids	Wagner's reagent	Reddish brown precipitates	Absent	Absent
	Dragendroff's reagent	Reddish brown precipitates	Absent	Absent
Phenolic	Ferric chloride	Dark green color	Present	Present
Tannins	Gelatin test	White precipitation	Absent	Absent
Steroids	Hesse's test	Pink color	Absent	Absent
Carbohydrates	Fehling's test	Green color suspension or red precipitate	Absent	Present
Saponin	Water test	Soapy forming substance	Absent	Present
Glycosides	Keller-killiani	Blue color	Absent	Present
Terpenoids	Salkowski	Layer of yellow color	Absent	Absent
Fats and Fixed oil	Stain test	Oil spot	Absent	Absent

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3.2 In-vitro Antioxidant Activity:

3.2.1 Total Phenol Content:

Antioxidants known as phenolic chemicals may break cycle of oxidation. Because of their hydroxyl groups, phenols are prominent plant components & may directly contribute to antioxidative action. As an indicator of total phenolic content, Folin-Ciocalteu reaction and gallic acid were employed to measure Clerodendrum colebrookianum Walp leaves hydroalcoholic or chloroform extracts. Both chloroform and hydro-alcohol were found to have 360.81mg/gallic acid equivalent (GAE) of Clerodendrum colebrookianum Walp leaves total phenol content. Consequently, total phenol content of hydro alcohol extract is larger than that of the chloroform (Fig.1).

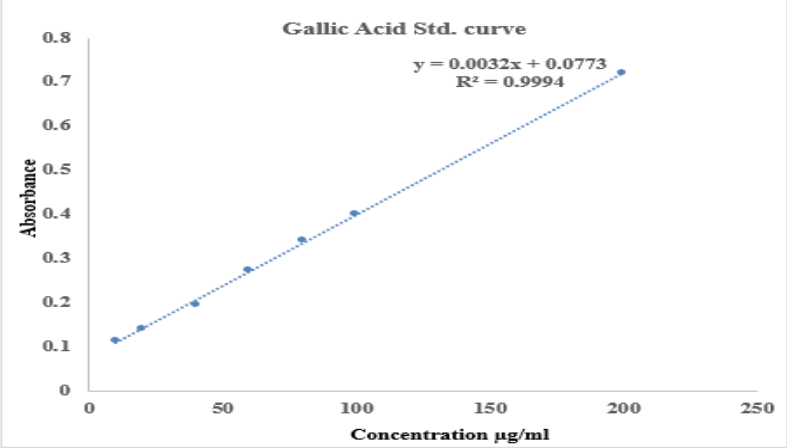

Fig.1: Gallic Acid Standard Curve

3.2.2 DPPH Scavenging Activity:

The stable radical DPPH was used to test Clerodendrum colebrookianum Walp leaves hydro alcohol and chloroform extract for their capacity to scavenge free radicals (2, 2-diphenol-2-picryl hydrazyl). Chloroform and hydro alcohol extracts of Clerodendrum colebrookianum Walp leaves 5g/ml had DPPH scavenging activities of 53.710.10 percent and 66.890.25 percent, respectively. Citric acid was found to have an IC50 of 1.748 milligrammes per millilitre (mg/ml), while chloroform & hydroalcoholic extracts of Clerodendrum colebrookianum were 4.46 and 3.33 milligrammes per millilitre, respectively (Fig.2).

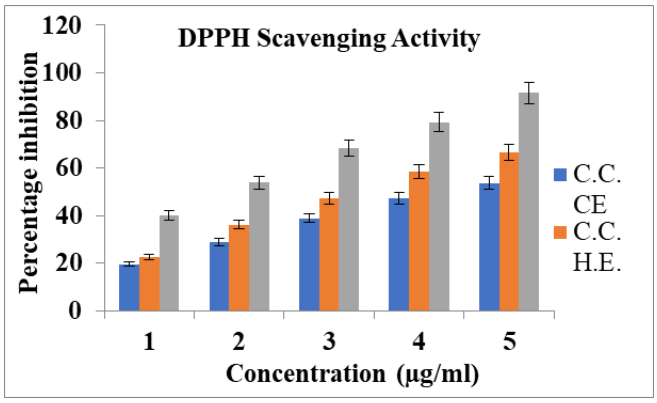

Fig.2: DPPH Scavenging Activity of Clerodendrum colebrookianum Walp leaves Extracts

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3.2.3 Hydrogen Peroxide Scavenging Activity:

Cells create hydroxyl radicals as a result of hydrogen peroxide. These radicals are utilised as an indicator of the extract's ability to neutralise them. It was revealed that chloroform and hydro-alcoholic extract of Clerodendrum colebrookianum Walp leaves had 74.5610.43 and 78.6971.14 percent antioxidative activity against hydrogen peroxide radicals, respectively, whereas the ascorbic acid concentration was 96.2200.03 percent. To determine the half-life of ascorbic acid, the researchers used 45.91 g/ml, 167.79 for the chloroform extract, and 189.92 for the hydro-ethanolic extract of Clerodendrum colebrookianum Walp leaves as their IC50 values (Fig.3).

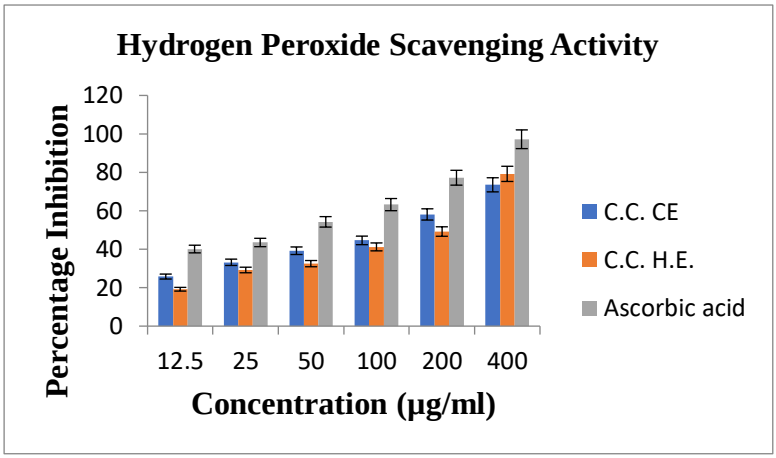

Fig.3: H2O2 scavenging activity of Clerodendrum colebrookianum Walp leaves

3.2.4 Reducing Power Assay:

The reduction of (Fe +++) to (Fe ++) in the presence of the test extracts, which subsequently combines with ferric chloride to generate ferric-ferrous complex with a maximum absorption at 700nm, was used to determine the antioxidant's reductive power. $EC_{50}$of chloroform and hydro-alcohol Clerodendrum colebrookianum Walp leaves was 18.54 and 233.55 µg/ml respectively & 19.56µg/ml for ascorbic acid (Fig.4).

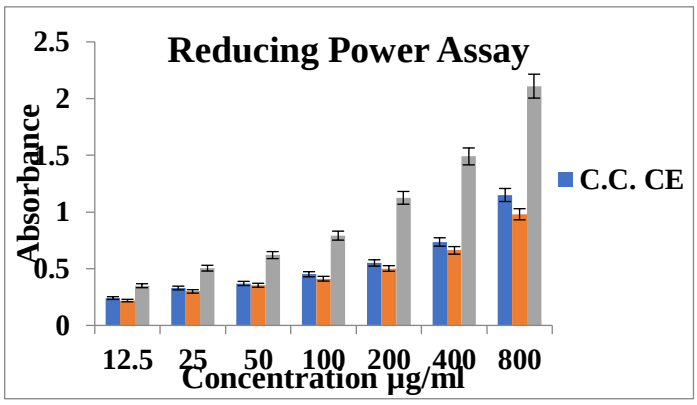

Fig.4: Reducing Power Assay of Clerodendrum colebrookianum Walp leaves Extracts

3.2.5 Superoxide Scavenging Activity

Clerodendrum colebrookianum Walp leaves extracts in choloroform and hydro-alcoholic form were shown to have antioxidant activity against superoxide dismutase of 72.3250.47 and 58.5390.17, respectively. Ascorbic acid's IC50 was 32.02g/ml, whereas chloroform extract's was 221.99 g/ml and hydro-alcoholic extract's was 288.18g/ml. (Fig.5).

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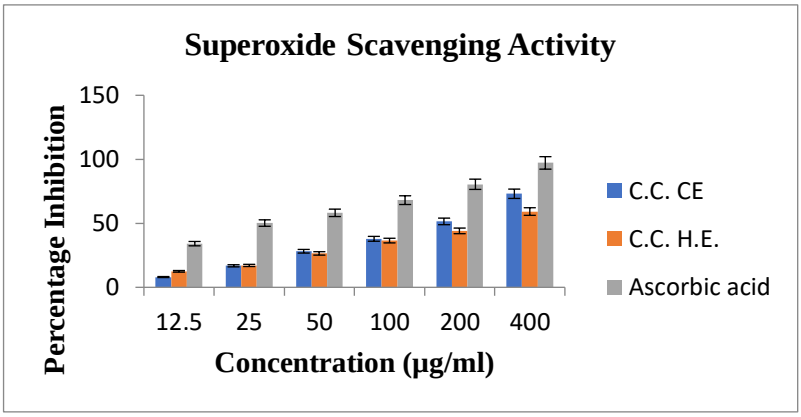

Fig.5: Superoxide Scavenging Activity of Clerodendrum colebrookianum Walp leaves Extracts

3.2.6 In-vivo Anti-platelet activity:

3.2.6.1 Thrombosis of Tail:

Carrageenan intravenous infusion caused swelling and redness within 2-3 hours, and the tail developed crimson patches after 6 hours, suggesting tail thrombosis. C.C. extract, C.H. extract, and hydro-alcoholic extracts all had a significant effect on the length of the infracted region in the rat tails of the responding rats, with C.C. extract having the greatest effect on the length of the infracted region in the tails of the responding rats, while C.H. extract had a greater effect on length than C.C. extract (Fig.6).

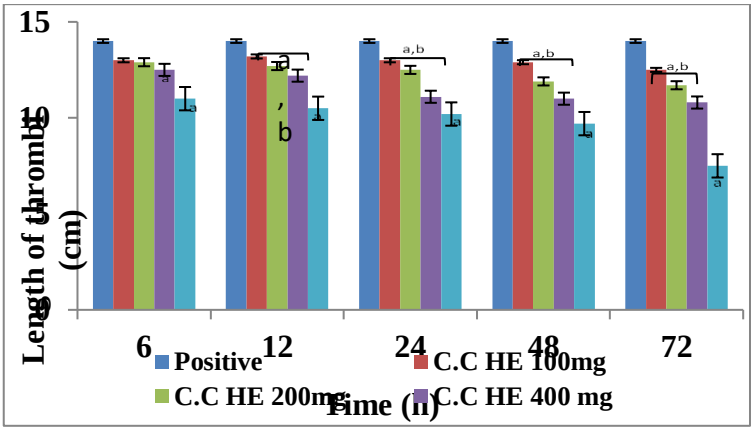

Fig.6: Effect of Clerodendrum colebrookianum Walp leaves hydro-alcoholic extract on progression of rat tail thrombosis.

3.2.6.2 Bleeding Time & Clotting Time:

Clerodendrum colebrookianum When Ivy's bleeding time technique and plain slide methods were used to the animals, the chloroform and hydro-alcoholic extract of wamp leaves showed a significant improvement in both the bleeding and clotting times. Bleeding and clotting times were significantly different in rats treated with chloroform and hydro alcohol extracts of Clerodendrum colebrookianum Walp leaves compared to rats treated with normal saline and the gold standard treatment, heparin. Clerodendrum colebrookianum Walp leaf extracts at 200mg/kg and 400mg/kg were substantially (p0.05) slower than conventional saline treatment in stopping bleeding. By spacing out your dosages farther, you may delay the rate at which your body loses blood.

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The results were the same after accounting for clotting time (Fig.7).

3.2.6.3 Tail Bleeding Assay with Drabkin's reagent:

We used the Drabkin's reagent tail bleeding test to determine haemoglobin levels after amputation. The compounds were administered orally to the animals after they had been fasted for an hour and weighed. One hour after birth, the animals were put to sleep with 100mg/kg of ketamine. After cutting the tail's distal 5 cm vertically at a 90° angle, 5 cc of Drabkin's reagent was used to collect the blood (Fig.7). Haemoglobin that has been lysed by this chemical reacts with potassium cyanide and potassium ferricyanide in an irreversible manner. When haemoglobin (Hb) is oxidised, cyanmethaemoglobin (a stable colour) is produced. It took 30 minutes to transfer the gently homogenised test tube five times to five different 100-ml test tubes (500 l each tube). The Drabkin's reagent volume was adjusted to 1.5ml after each transfer. Absorption spectroscopy was used to quantify the concentration of haemoglobin (Hb) in the blood. Chloroform and hydro alcohol extracts of the Clerodendrum colebrookianum Walp leaves were able to impact the haemoglobin mass loss at each 5-minute time interval, as contrasted to the value obtained from rats treated with control group normal saline or standard therapy. One-way ANOVA and Tukey's Multiple Range Test were used to examine the data collected from the different study groups. Statistical significance is defined as p 0.05. Comparing a,b: those treated with extract to those treated with normal a,c: In the graph, (Group Normal=0 is not shown)

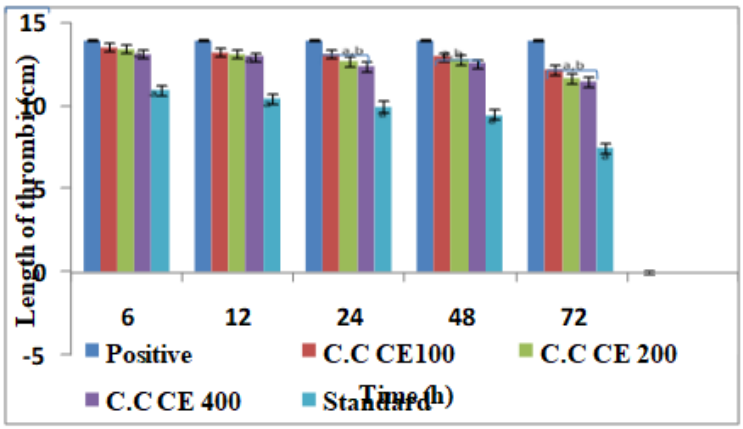

Fig.7: Effect of Clerodendrum Colebrookianum chloroform extract on progression of rat tail thrombosis.

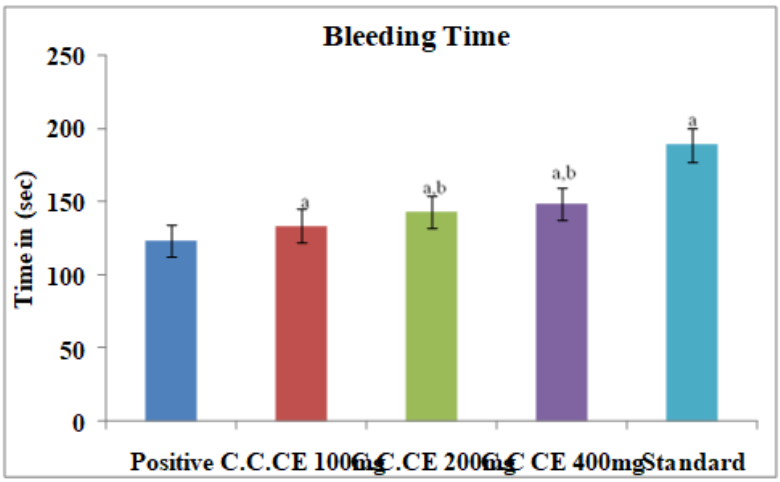

Fig.8: Effect of Clerodendrum Colebrookianum chloroform extract on bleeding time.

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Data from several groups were analysed using one-way ANOVA & Tukey's Multiple Range Test. Results are presented as mean and standard error of mean (SEM). Comparing standard and extract treatment groups in terms of sample size.

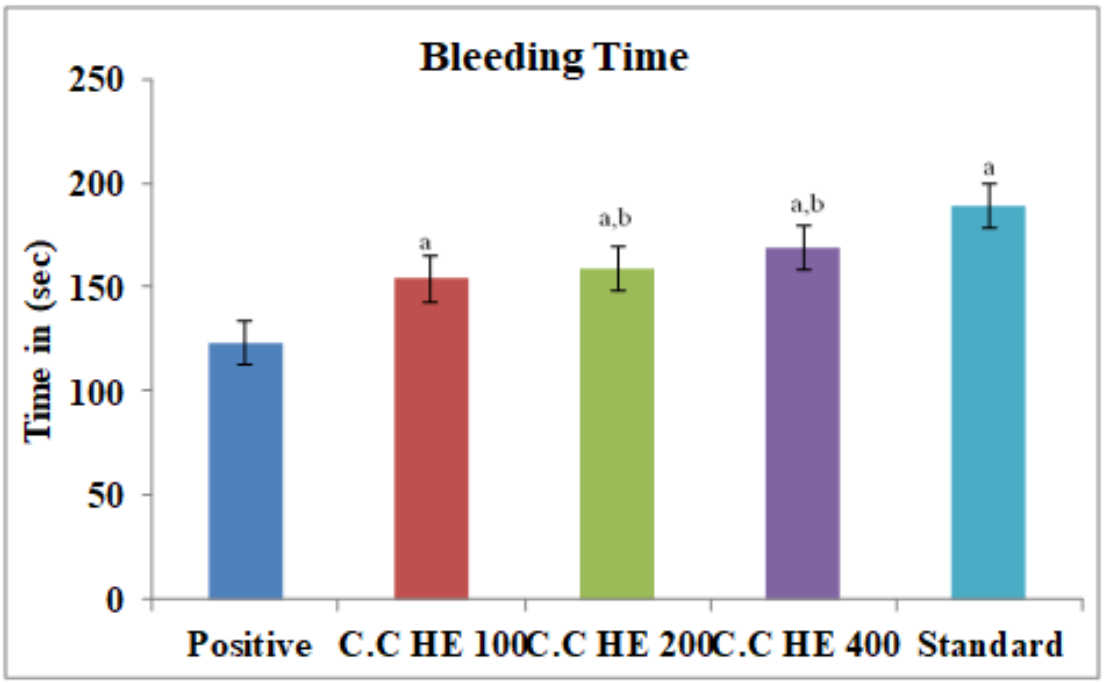

Fig.9: Effect of Clerodendrum Colebrookianum hydro-alcoholic extract effect on bleeding time.

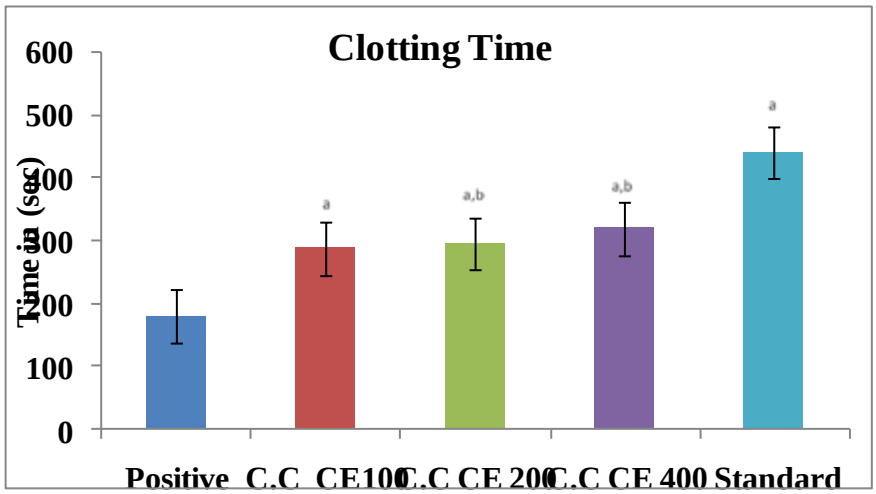

Fig.10: Effect of Clerodendrum Colebrookianum chloroform extract on clotting time.

Tukey's many Range Test and a one-way analysis of variance were used to compare the means of many groups. Mean and standard error of mean (SEM) are shown for the results. Conventional treatment (c) was compared to the control group (a) and the extract-treated group (b).

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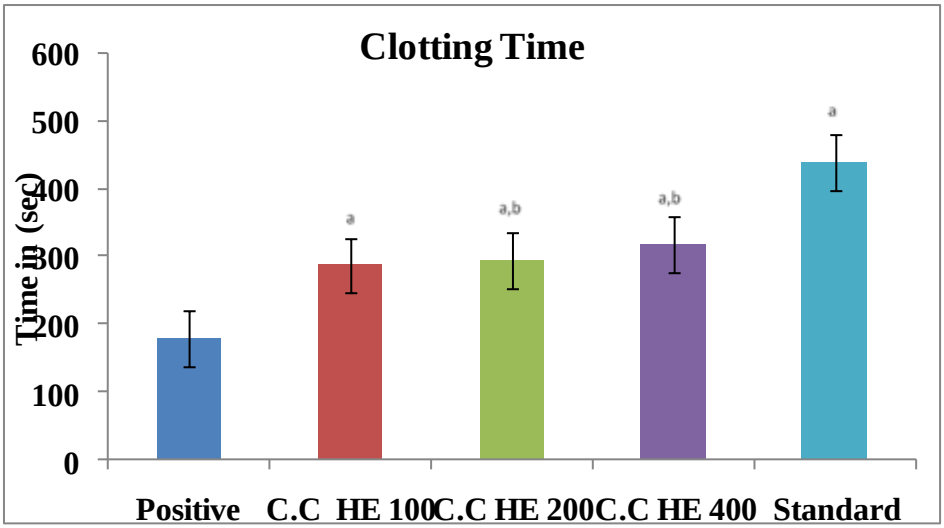

Fig.11: Effect of Clerodendrum Colebrookianum hydro-alcoholic extract on clotting time.

To compare data from various categories, we utilised one-way ANOVA and Tukey's Multiple Range Test. The results are shown as means SEM, and significance was considered at the p0.05 level. Taking a look at how the standard treatment group stacks up against the extract group

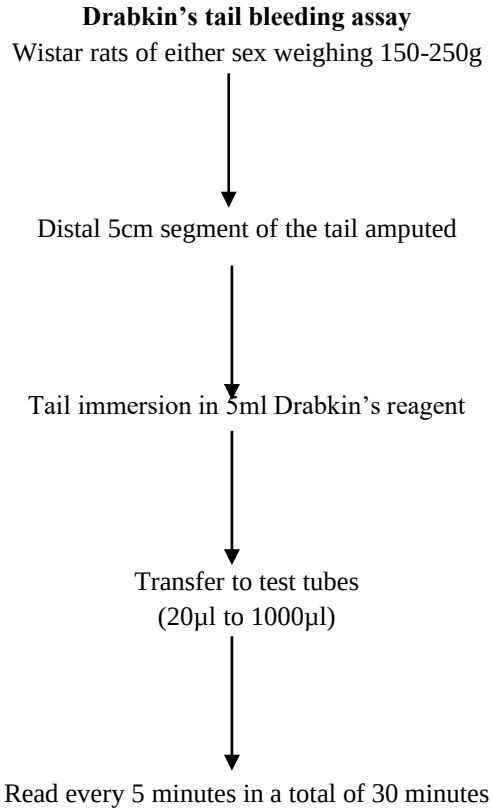

Fig.12: Drabkin's tail model assay

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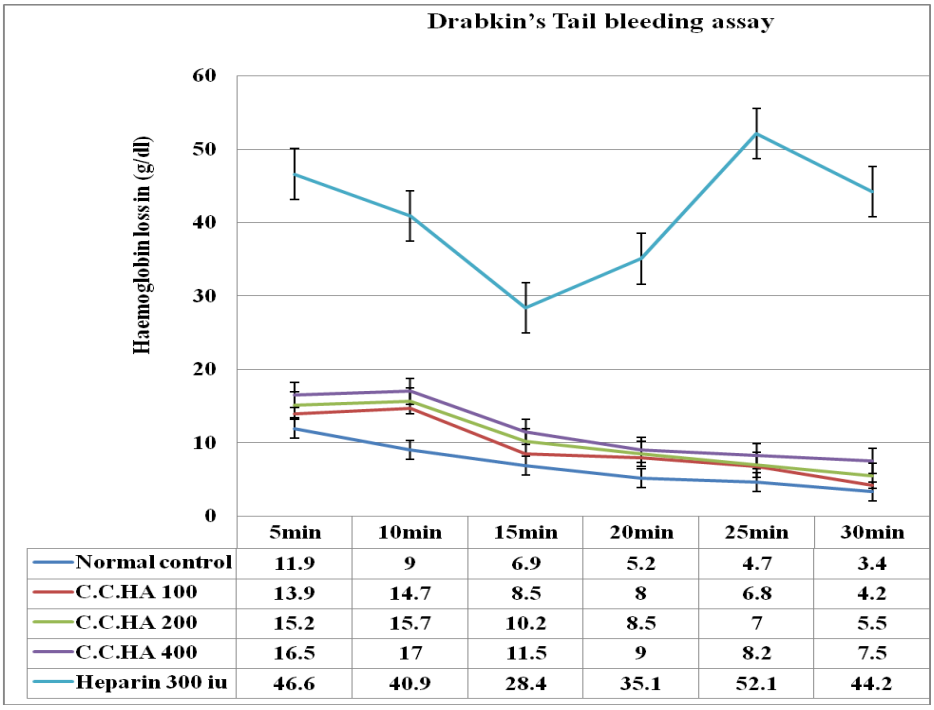
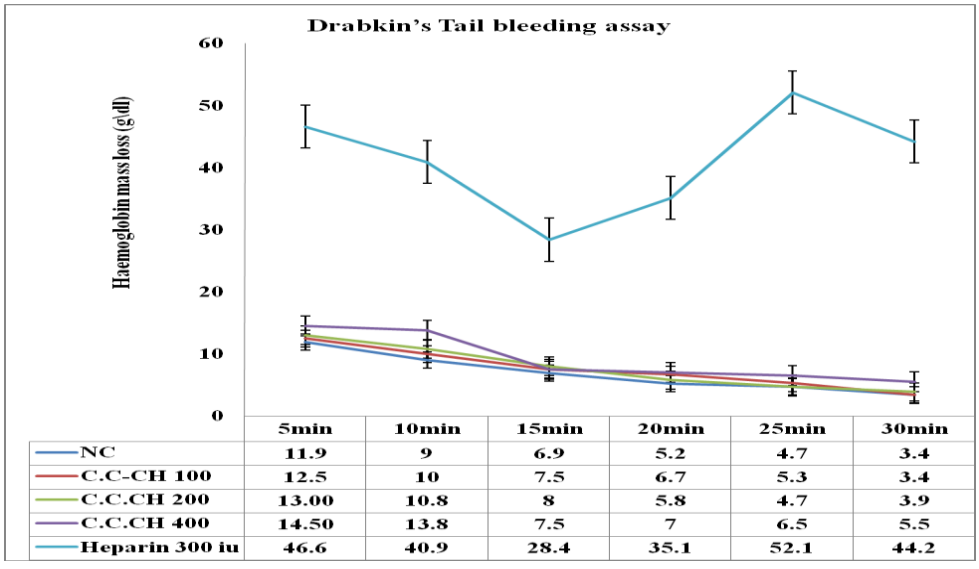


	5min	10min	15min	20min	25min	30min
Normal control	11.9	9	6.9	5.2	4.7	3.4
C.C.HA 100	13.9	14.7	8.5	8	6.8	4.2
C.C.HA 200	15.2	15.7	10.2	8.5	7	5.5
C.C.HA 400	16.5	17	11.5	9	8.2	7.5
Heparin 300 iu	46.6	40.9	28.4	35.1	52.1	44.2

Fig.13: Comparing the anti-coagulant and anti-platelet activities of Clerodendrum Colebrookianum extract with Heparin treated and NC normal control and hydro-alcoholic extract using various time tests and haemoglobin concentrations.



	5min	10min	15min	20min	25min	30min
NC	11.9	9	6.9	5.2	4.7	3.4
C.C-CH 100	12.5	10	7.5	6.7	5.3	3.4
C.C.CH 200	13.00	10.8	8	5.8	4.7	3.9
C.C.CH 400	14.50	13.8	7.5	7	6.5	5.5
Heparin 300 iu	46.6	40.9	28.4	35.1	52.1	44.2

Fig.14: Comparing the anti-coagulant and anti-platelet activities of Clerodendrum Colebrookianum extract with Heparin treated and NC normal control and chloroform extract using various time tests of haemoglobin concentration

Conclusion:

Experiments were conducted in this work to examine the pharmacological effects of several Clerodendrum colebrookianum walp extracts on antithrombotic activity. There was significant antithrombotic and anti-platelet activity in the chloroform and hydro alcohol (40 percent) extracts of Clerodendrum colebrookianum walp, including flavonoids, sterols, saponins, alkaloids, glycosides, phenols, fatty acids and carbohydrates, all of

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which are well-known for their role in the treatment of thrombosis and its complications. Flavonoids and Polyphenols are plant-based chemicals that naturally exist in the human body. Antithrombotic action has been linked to a number of flavonoids. These elements may be responsible for these effects in this study. In the Drabkin's tail model experiment in vivo, it has favourable benefits on lowering the length of thrombi and re-bleeding activity. Clerodendrum colebrookianum walp showed promising and efficient antioxidant benefits in in-vitro. Clerodendrum colebrookianum walp, an ethno-medicinal plant, has been shown to have great therapeutic potential. However, more comprehensive research on each Phytoconstituent is required if plant activity is to be improved for future therapeutic usage.

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