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Evaluation of in vitro Anticoagulant Activity of *Camellia sinensis*

[#]Department of Chemistry, VivekanandCollege, Aurangabad (Corresponding Author).

Adhyapak M.S[#]. and kachole M.S.[*]

[*]Department of Biochemistry,Dr.Babasaheb Ambedkar Marathwada University, Aurangabad.

Abstract:

Most of the current available anticoagulant drugs have certain limitations/undesired effects in case of some patients. Attempts have been made to discover new anticoagulant drugs of plant origin. The anticoagulant activity of *Camellia sinensis*was evaluated in vitro, wherein, aqueous, methanol and ethanol ext showed weak anticoagulant activitybyforming partial clots. The activity was retained even up to 20% dilution and showed strong activity with no clot formation after heat treatment. This evaluation could help in the use of *Camellia sinensis*as a preventive herbal anticoagulant drug.

(**Keywords**: Anticoagulant activity, cardiovascular disease, clotting time, *Camellia sinensis*)

Introduction:

Cardiovascular disease is a leading cause of death throughout the world. This disease is caused mainly due to the abnormal blood coagulation (clotting) in the arteries supplying blood to the heart. Blood clots that develop in the arteries can cause heart attack/stroke[1]. The clots cut off blood flow to the heart[2].The undesired blood clot interferes in the free flow of blood leading to dysfunction/permanent damage to the heart. The normal coagulation process is essential to avoid excessive blood loss through the damaged blood vessels; but undesired blood coagulation results in several life threatening diseases. Formation of blood clots in the arteries supplying blood to the heart or brain is the common cause of heart attack and stroke[3]. The clotting tendency is treated by the use of anticoagulant drugs such as heparin and warfarin which have been in use from about fifty years[4].

Although heparin and warfarin have been proved as effective anticoagulants, recent studies have revealed some limitations/adverse effects of both the drugs[5-13].Bleeding and

alopecia are the major side effects of heparin[14]. Warfarin requires frequent monitoring and has several adverse effects[15-16] like bleeding and adverse skin manifestations[17-18]. Background intracranial haemorrhage is the most feared and lethal complication of oral anticoagulation[19].One of the most important adverse effects is life-threatening haemorrhage[20-21].

Limitations of existing anticoagulants have accelerated a search for new anticoagulants with improved pharmacological and bio-safety profile[22]. At present, the greatest clinical need is for an oral anticoagulant to replace warfarin for long term prevention and treatment of patients with venous and arterial thrombosis[23].

Plants produce a variety of phytochemicals/natural products. Natural products have been found to be a significant source of commercial medicines[24]. Proteases represent a class of enzymes having physiological roles[25]. Protease inhibitors are natural products of plants[26]. They are anti-metabolic proteins which interfere with the digestive process of insects[27]. They are plants' defence machinery against predators and pathogens[28-30]. Protease inhibitors have broad spectrum of biological activites[31].

There is a growing focus on the importance of medicinal plants in the traditional health care system[32]. Plant extracts can be used as a tool in the development of plant derived drugs[33]. Natural compounds have practical advantage that they are suitable for oral applications[34].

It is reported that, *Camellia sinensis* shows anticoagulant activity[35-36]andhence, it can be used as preventive anticoagulant drug. In the present study, the anticoagulant activity of *Camellia sinensis* is evaluated in vitro.

Materials and method:

Human blood was collected(having no medicine consumption history) by making venipuncture. To the 9 ml volume of blood, 1 ml of 3.8 % tri-sodium citrate (prepared in 0.85% saline solution) was added to avoid natural coagulation. The powdered form of leaves of *Camellia sinensis* was purchased from local market. Tri-sodium citrate was purchased from Sisco Research Lab.

The extract of *Camellia sinensis*was prepared by dissolving 1g powderedleaf part in the solvents- water, methanol, ethanol, ethyl acetate and hexane, 5 ml each. The exts were centrifuged at 10000 rpm for 20 min.

Blood and plant exts were mixed with 100:100µlproportions. The clotting time was measured after addition of 100 µl $CaCl_2$ (0.025 moles/L) into the blood–plant ext mixture, using Lee and White method[37].The clotting status was examined continuously by tilting the sample holders containing test and control simultaneously at regular intervals. The control was prepared by mixing100 µl volume of blood with 100 µl solvent. The aqueous ext was diluted from 10 to 90% and the activity of each dilution was tested in vitro. The aq ext was heated up to boiling. The clotting test was also carried for the boiled ext.

Results and Discussion:

Solvent	**Clotting Status**	
	Control	***Camellia sinensis***
Water	C	P
Methanol	C	P
Ethanol	C	P
Ethyl acetate	C	C
Hexane	C	C

(C - Clotted, P - Partially clotted)

Table 1.1: Response of plant extracts to clotting time test in various solvents

Formation of clot was seen in the control after seven min, while the water, methanol and ethanol ext of *Camellia sinensis* showed partial clotting status suggesting weak activity. The ethyl acetate and hexane ext showed full clot formation indicating zero activity.

Blood Volume (µl)	**Ext + Solvent Proportion** (µl)	**Clotting Status**
100	00+100 (Control)	C
100	100+00	P
100	90+10	P
100	80+20	P
100	70+30	P
100	60+40	P
100	50+50	P
100	40+60	P
100	30+70	P
100	20+80	P
100	10+90	C

(C - Clotted, P - Partially clotted)

Table 1.2: Clotting status of *Camellia sinensis* at various dilutions

When diluted, the aq ext of *Camellia sinensis*retained its activity up to 20% dilution.

Sample Ext	Clotting Status	
	Before Heat Treatment	After Heat Treatment
Control	C	-
Camellia s.	P	N

(C - Clotted, P - Partially clotted, N- Not clotted)

Table 1.3: Clotting status after heat treatment

Effect of heat treatment- When heated up to boiling, the aq ext did not show clot formation for a prolonged period of time, which suggests presence of strong anticoagulant activity in the boiled ext.

This indicates that the aq ext, though showed weak anticoagulant activity, is suitable for oral application which could be an alternative/supportive, in future, to the current synthetic drugs. Low conc of the ext, as low as 20%, is also sufficient to show the positive activity. This may help in deciding the dose quantity for prevention/treatment purpose. The activity was increased after heat treatment;hence we recommend using the ext in boiled state to get maximum health benefits.

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