

# ***In vitro* Studies on Antibacterial, Thrombolytic and Antioxidant Activities of Green Tea or *Camellia sinensis***

**Mir Monir Hossain\* and Shahrin Mahmood**

Department of Pharmacy, University of Science & Technology Chittagong (USTC) Foy's Lake, Chittagong-4202, Bangladesh

## **ABSTRACT**

This study examined the antibacterial, thrombolytic and antioxidant activity of crude water, ethanol and acetone extracts of *Camellia sinensis* (Fam: Theaceae) or green tea leaves *in vitro*. The pharmacological history of this plant inspired us to evaluate the possible antibacterial, thrombolytic and antioxidant potentials. A quick and rapid methodology (*In vitro* thrombolytic model) was applied to find out the clot lysis effect of aqueous, ethanol and acetone extracts of *Camellia sinensis* leaves where streptokinase and water were employed as a positive and negative controls, respectively. The percentage (%) clot lysis was statistically significant ( $p < 0.0001$ ) when compared with vehicle control. Using an *in vitro* thrombolytic model, water, ethanol and acetone extracts of *Camellia sinensis* leaves showed moderate clot lysis activity ( $45.60 \pm 2.313\%$ ,  $37.68 \pm 2.211\%$ , and  $30.51\% \pm 2.551\%$ , respectively) whereas standard streptokinase showed  $87.15 \pm 3.212\%$  clot lysis effect and the negative control water revealed  $5.60 \pm 2.131\%$  lysis of clot. In the case of antibacterial activities, the extracts of water, ethanol and acetone solvents were investigated by a simple agar diffusion method using ten pathogenic bacteria. Both aqueous and ethanol extracts showed moderate activity against seven pathogenic organisms, and acetone extracts showed activity against eight of the bacteria tested. All the activities were determined by measuring the zone of inhibition (in mm) compared with the standard antibiotic (Amoxicillin). Finally, Free radical scavenging activity was evaluated *in vitro* with the spectrophotometric method based on the reduction of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical. The tested extracts showed variable antioxidant activities. The high antioxidant activity was achieved in water (88.32%) followed by ethanol (78.80%) and acetone (61.96%) extracts. The extracts were investigated regarding their total flavonoid content (TFC) by  $\text{AlCl}_3$  reagent. The aqueous extracts of green tea leaves (TFC =  $78.16 \pm 1.90 \mu\text{g}/\text{mg}$ ) and ethanol extracts (TFC =  $65.99 \pm$

## **Address for Correspondence**

Department of  
Pharmacy University  
of Science &  
Technology  
Chittagong (USTC)  
Foy's Lake,  
Chittagong-4202,  
Bangladesh.

E-mail: [monir\\_pharm@yahoo.com](mailto:monir_pharm@yahoo.com)

4.11 µg/mg) had the highest content of flavonoid in comparison to acetone extracts (TFC = 55.85 ± 1.43 µg/mg). Furthermore, the total phenolic content of the three extracts of green tea leaves showed large variations. The water extracts of green tea leaves contained the highest total phenol content (160.00 ± 0.62 mg GAE/g extract), followed by ethanol extracts (123.33 ± 2.32 mg GAE/g extract) and acetone extracts (95.37 ± 1.12 mg GAE/g extract). This study suggests that leaves of green tea are the possible sources of natural radical scavengers. Thus, green tea leaves could be used as natural antioxidants in the beverage, food and pharmaceutical industries that need further wide range *in vivo* studies.

**Keywords:** *Camellia sinensis*, Antibacterial activity, Agar diffusion method, Zone of inhibition, Thrombolysis, Antioxidant activity, DPPH assay.

## INTRODUCTION

Tea is produced from leaves and non-developed buds of a tea shrub having two botanical varieties: *Camellia sinensis* (Family: Theaceae) and *Camellia assamica*. Bangladesh is a tea (black tea) producer in the world followed by India (black tea), Japan (green tea) and China (different sorts of tea). Tea is the most consumed beverage in the world besides water. Based on the manufacturing process, there are three major types of tea: black (fully aerated or fermented); green (unaerated or unfermented) and oolong (partially aerated or semi fermented)<sup>1-3</sup>. Medicinal properties of tea were known to mankind since antiquity. Several studies have shown that tea, especially the black and green types, impair blood clotting in man and animals. Further, it is hypofibrinogenic and shown to inhibit platelet aggregation in rabbits both *in vivo* and *in vitro*<sup>1,2,5,6</sup>. As such, tea is claimed to be cardioprotective<sup>1,2</sup>. However, less attention has been focused on the clot lysing activity of tea. Since tea contains a variety of water soluble phytoconstituents<sup>1,7</sup>, it is possible that tea may affect thrombolysis.

Microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the common pathogens of human infection. *Staphylococcus aureus* is an opportunistic pathogen of human skin. *Pseudomonas aeruginosa* is a pathogen associated with pyogenic infection and urinary tract infection. These microorganisms are highly pathogenic and rate of infection caused by these microorganisms are considerably increasing in recent years. Hence the use of plant products has been increasing worldwide, to minimize side effects<sup>8</sup>. Green tea is generally safe, non toxic and having no side effects after use. However, over consumption may cause in treating infection, human disease because to associated to lower side effects<sup>9</sup>.

Antibacterial property of tea was first reported from Japan by using Japanese tea against various diarrheal pathogens<sup>10</sup>. Subsequent studies with four kinds of Japanese green tea and 24 bacterial isolates from infected root canals provided enough evidences to support the bactericidal activity of tea<sup>11</sup>. Extracts of tea have shown significant bactericidal activity against

methicillin resistant *Staphylococcus aureus* (MRSA) even at concentrations available in ordinarily brewed tea<sup>12</sup>. There have been studies conducted in the past to evaluate the antibacterial activity of tea against *Salmonella* serotypes primarily associated with diarrheal illness<sup>13</sup>. However, there is a paucity in the information available, regarding the antibacterial activity of black tea extracts against serotypes of *Salmonella* causing enteric fever.

The large generation of free radicals, particularly reactive oxygen species and their high activity plays an important role in the progression of a great number of pathological disturbances like inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson's disease, Alzheimer's disease, etc<sup>14-17</sup>. Therefore, the great interest has been recently focused on the natural foods, medicinal plants and phytoconstituents due to their well-known abilities to scavenge free radicals (i.e. antioxidant power)<sup>18,19</sup>.

Tea has been used as a daily beverage and crude medicine throughout the world for thousands of years. Tea possesses antipyretic and diuretic effects, etc. The pharmacological effects of tea are reviewed, including antioxidative activity<sup>20</sup> and antimutagenic<sup>21-23</sup> and anticancer effects<sup>24</sup>. Concerning the antioxidative and anticarcinogenic effects of tea, researchers reported that green tea antioxidant (GTA) had antioxidative activity toward hydrogen peroxide and superoxide and that GTA prevented oxygen radical and hydrogen peroxide induced cytotoxicity and inhibition of intercellular communication in cell culture<sup>25</sup>.

Thus, the aim of this study was to determine the thrombolytic potential, antibacterial and antioxidant activities of green tea *in vitro*.

## MATERIALS AND METHODS

### Collection of black tea leaves

The aerial parts of wild-growing *Camellia sinensis* were collected at their fully mature forms, from different tea gardens of Moulovibazar, Sylhet, northeast part of Bangladesh at an altitude of 220 m. The parts of plants were identified by the Bangladesh Council of Science and Industrial Research (BCSIR), Chittagong, Bangladesh. Samples were pure without any aromatic or additive materials. Various extracts of the leaves were prepared in different solvents, and then these were tested for antibacterial activity against ten pathogenic bacteria, and finally evaluated the thrombolytic potential and antioxidant activity.

### Preparation of plant extracts

Fresh plant leaves were washed under running tap water and ethanol (30-40%). The leaves were cut into pieces and ground into fine powder with an electric grinder. The powder was stored in air tight bottle<sup>26</sup>. Aqueous extract was prepared by mixing 20.0gm of dry powder of plant leaves with 200 ml. of sterile distilled water in a round bottom flask (no.72) with occasional shaking. Before placing, the flask was washed properly and then dried. The extract was then filtered through a muslin cloth for coarse residue and finally filtered through Whatman No.1 filter paper with 150µm diameter and stored in an airtight container at 4°C until use<sup>27</sup>. Ethanolic extract was prepared by mixing 50.0gm of dry powder of plant leaves with 200 ml. of 99% ethanol (Merck, Germany) and kept at room temperature for 7 days in a round bottom flask with occasional shaking. After a seven day period, the extract was filtered through a muslin cloth for coarse residue and final filtration was done through Whatman No.1 filter paper and stored in airtight bottle at 4°C until use<sup>28</sup>. In the same

way the acetone extract was prepared by mixing 50.0gm of dry powder of plant leaves with 200 ml. of 99% acetone (Merck, Germany) and kept at room temperature for 7 days in a round bottom flask with occasional shaking. After a seven day period, the extract was filtered through a muslin cloth for coarse residue and final filtration was done through Whatman No.1 filter paper and stored in airtight bottle at 4°C until use<sup>29</sup>.

#### *In vitro* antibacterial assay

Study of *in vitro* antibacterial activity of the aqueous, ethanol and acetone extracts obtained from the extraction of the leaves of the plant *Camellia sinensis*. Ten pathogenic bacteria were used as test organisms for antibacterial activity of the dried extracts. All extracts were tested for antimicrobial study by using standard disc diffusion method<sup>30,31</sup>. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in millimeters) of bacterial growth<sup>32-34</sup>.

The bacterial strains were collected from the microbiology laboratory of BCSIR, Chittagong, Bangladesh. Nutrient agar media was used for the culture of the test organisms and the antibacterial activity was determined by single disc diffusion method<sup>35</sup>.

Nutrient agar medium (23 gm) was suspended in 1000 ml of water and heated to make a clear solution. Then from this clear solution concentrated agar plates were prepared. The standard discs 0.1mg Amoxicillin/disc was used to compare the activities of test samples. For extracts 0.5mg/disc samples were used. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates, pre-inoculated with the test organism. The discs were then incubated on the plate aerobically at 37°C for 24 hours. The

diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period. The extract concentration, ability to inhibit microbial growth, which was observed through the formation of an inhibition growth zone around the disc (equal to or greater than 8 mm)<sup>36</sup>, was considered.

#### *In vitro* thrombolytic activity

##### Herbal preparation

100 mg of each extract was suspended in 10 ml distilled water separately and then the suspensions were shaken vigorously on a vortex mixer. The suspensions were kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. All solutions were then ready for *in vitro* evaluation of clot lysis activity<sup>37</sup>.

##### Streptokinase (SK)

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis<sup>37</sup>.

##### Specimen

Whole blood (5 ml) was drawn from healthy human volunteers (n = 10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots<sup>37</sup>.

##### Clot lysis study

Experiments for clot lysis were carried as reported earlier<sup>37</sup>. Venous blood was drawn from healthy volunteers (n = 10) and transferred in different pre-weighed

sterile alpine tube (500 µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of the tube alone). Each alpine tube containing clot was properly labeled and 100 µl of different plant extracts was added to the tubes separately. As a positive control, 100 µl of Streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated several times with the blood samples of different volunteers.

$\% \text{ clot lysis} = (\text{Weight of the lysis clot} / \text{Weight of clot before lysis}) \times 100$

### Statistical analysis

The significance of % clot lysis by herbal extracts by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean  $\pm$  standard deviation.

### *In vitro* antioxidant potential

#### Chemicals

All of the chemicals used in this work were purchased from Merck (Germany), with the exception of DPPH, Flavonoid standards, gallic acid, BHA (tertbutyl-4-hydroxy-anisol), and  $\alpha$ -tocopherol, those were purchased from Sigma-Aldrich (St. Louis, MO, USA). The chemicals were analytical grade.

### Free radical scavenging activity

The free radical scavenging activity of the extracts was estimated according to the procedure described by researchers<sup>38,39</sup>. Briefly, a 0.3mM solution of the DPPH radical solution in ethanol 90% were prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations of each extract (sample). The mixture was shaken and left for 30 min at room temperature in the dark, and after 30 min incubation the absorbance was then measured with a spectrophotometer at 517 nm. The antioxidant activity was calculated as the percentage of the radical scavenging activity (RSA) by the following equation:

$$\text{RSA}\% = [\text{Acontrol} - (\text{Asample} - \text{Ablank})] / \text{Acontrol} \times 100$$

Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. Rutin solution (at the concentrations of 100, 50, 25, 10, 5, 2.5µg ml<sup>-1</sup>) was used as a positive control.

The IC<sub>50</sub> value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the nonlinear regression curve of Log concentration of the test extract (µg ml<sup>-1</sup>) against the mean percentage of the radical scavenging activity.

### Total flavonoid content (TFC)

The total flavonoid content in extracts was determined according to researchers<sup>38</sup>. Briefly, 2.5 ml of each extract solution was mixed with 2.5 ml AlCl<sub>3</sub> reagent in ethanol 90% and allowed to stand for 40 min for reaction at room temperature. After that, the absorbance was measured spectrophotometrically at 415 nm. Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound.

The TFC for each extract [as µg rutin equivalents (RE) /mg of extract] was determined on the basis of the linear calibration curve of rutin (absorbance versus rutin concentration).

#### Total phenolic content (TPC)

The extraction of total phenolics was performed using the Folin–Ciocalteu assay, following a standard method with some modifications<sup>40</sup>. In total, 100 µl of each extract (1 mg/ml) was added to a test tube containing 50 µl of the phenol reagent (1M). A further 1.85 ml of distilled, deionized water was added to the solution and allowed to stand for 3 min after vortexing. Then 300 µl Na<sub>2</sub>CO<sub>3</sub> (20% in water, v/v) was added and vortexed, and the final volume (4 ml) was obtained by adding 1.7 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The final mixture was vortexed, then incubated for 1 h in the dark at room temperature. The absorbance was measured at 725 nm using a UV-VIS spectrophotometer. A standard curve was prepared using 0, 65.5, 125, and 250 mg/l gallic acid in methanol: water (50:50, v/v). Total phenolic values are expressed in terms of gallic acid equivalents (GAE) in milligrams per gram plant extract. All determinations were performed in triplicate.

#### Statistical analysis

All of the experiments were carried out in triplicate. Antioxidant activity, total phenolic content, and flavonoid content are reported as the mean ± standard deviation (SD). Significant differences for multiple comparisons were determined using one way analysis of variance (ANOVA). Duncan's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA).

Differences at  $P < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

#### *In vitro* antibacterial study

The extracts of the sample were tested for antibacterial activity against ten pathogenic both gram-positive and gram-negative bacteria. Standard antibiotic disk of amoxicillin was used for comparison purposes. The three different extracts of the leaves of the black tea showed moderate antibacterial activity against some of the test organisms. The results of the antibacterial activity, measured in terms of the diameter of zone of inhibition in mm are shown in table 1. The zone of inhibition was found in different organisms.

From the table 1, it is observed that, the water extract showed moderate antibacterial activity against some test organisms, namely- *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Shigella dysenteriae*, *Escherichia coli*, and *Bacillus megaterium*. The ethanol extract showed activity against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Shigella dysenteriae*, *Staphylococcus pyogenus* and *Escherichia coli*. On the other hand, the acetone extract gave activity against *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus cereus*, *Shigella dysenteriae*, *Staphylococcus pyogenus* and *Escherichia coli*. Again the standard sample (Amoxicillin) showed remarkable antibacterial activity against all tested bacteria.

#### *In vitro* thrombolytic study

As a part of discovery of cardio protective drugs from natural resources the different extracts of green tea or *Camellia sinensis* were assessed for thrombolytic activity. In this connection, Addition of 100 µl SK (Streptokinase), a positive control

(30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed  $87.15 \pm 3.212$  % lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentages of lysis of clot ( $4.50 \pm 1.110$  %). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant (p value  $<0.0009$ ). The *in vitro* thrombolytic activity study revealed that, water extract of Green tea leaves exhibited highest thrombolytic activity ( $45.60 \pm 2.313$ %). However, significant thrombolytic activity was demonstrated by the ethanol and acetone extracts showed  $37.68 \pm 2.211$ %, and  $30.51\% \pm 2.551$ %, respectively.

Statistical representation of the effective thrombolysis percentage by various solvent extracts of black tea leaves, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table 2.

Statistical representation of the effective thrombolysis percentage by different solvent extracts of green tea leaves, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; % thrombolysis is represented as mean  $\pm$  S.D. and p values of all solvent extracts were  $< 0.05$  was considered as significant.

% thrombolysis obtained after treating clots with different solvent extracts and appropriate controls can be shown in the following Figure 1.

#### DPPH assay for free radical scavenging potency

Antioxidant tests could be based on the evaluation of lipid peroxidation or on the measurement of free radical scavenging potency (hydrogen-donating ability). The radical scavengers donate hydrogen to free radicals, leading to non toxic species and therefore to inhibition of the propagation

phase of lipid oxidation. The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers<sup>41-43</sup>. The antioxidant activity of medicinal plants is mainly related to their bioactive compounds, such as phenolics, flavonols, and flavonoids.

In this study, the antioxidant capacity of water, ethanol and acetone extracts of green tea leaves was systematically evaluated. The DPPH inhibition of different plant extracts is summarized in Table 1. Water extracts of leaves of black tea possessed the highest DPPH scavenging activity ( $88.32 \pm 1.53$ % inhibition of the DPPH radical), followed by ethanol and acetone extracts ( $78.80 \pm 1.73$ % and  $61.96 \pm 1.53$ %, respectively) of green tea leaves, comparable to the commercial antioxidant BHA (93.03% inhibition of the DPPH radical) and  $\alpha$ -tocopherol (92.00%). The antioxidant activity of the leaves of *C. sinensis* could be due to the presence of a wide variety of bioactive compounds, such as phenolics, flavonoids, carotenoids, and tannins in this plant.

#### Total flavonoid content (TFC):

Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging properties<sup>44</sup>. The total content of flavonoids was evaluated and expressed as  $\mu$ g rutin equivalents (RE) /mg of extract. The content of flavonoids varied from  $14.85 \pm 1.43$  to  $58.11 \pm 1.90$   $\mu$ g/mg (Table 1). The highest amount of flavonoids was found in the water extracts of leaves of green tea ( $78.16 \pm 1.90$   $\mu$ g/mg), followed by ethanol extracts ( $65.99 \pm 4.11$   $\mu$ g/mg) and acetone extracts ( $55.85 \pm 1.43$   $\mu$ g/mg), indicating that these phytochemicals are likely to be responsible for the free radical scavenging activity. Flavonoids are reportedly responsible for the antioxidant activities of plants<sup>45</sup> through their scavenging or chelating activity<sup>46</sup>.

### Total phenolic content (TPC)

Phenolics are well established to show antioxidant activity and contribute to human health. In this study, the total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid as a standard. The content of phenolics was evaluated and expressed in GAE as milligrams per gram of extract (mg GAE/g extract). The total phenolic content of the three extracts of green tea leaves showed large variations. The water extracts of green tea leaves contained the highest total phenol content ( $160.00 \pm 0.62$  mg GAE/g extract), followed by ethanol extracts ( $123.33 \pm 2.32$  mg GAE/g extract) and acetone extracts ( $95.37 \pm 1.12$  mg GAE/g extract). The values of total phenolic content are shown in the table 3.

### CONCLUSION

The present study is designed to check antibacterial, thrombolytic and antioxidant activities of different solvent extracts of green tea or *Camellia sinensis* leaves. In antibacterial evaluation, the aqueous extracts showed antibacterial activity against seven pathogenic organisms, ethanol extracts also exhibited antibacterial activity against seven of the pathogenic organisms and acetone extracts showed activity against eight of the bacteria tested. As evident from this antibacterial study, leaves of *Camellia sinensis* may contain important chemical substances that confer upon this plant as a medicinal agent possessing antibacterial activity. This will help us to design chemotherapy against the disease cause of them. Again from this experiment, it can be concluded that the various solvent extracts of leaves of green tea showed moderate clot lysis activity. Once found, these tested extracts may be incorporated as a thrombolytic agent for the improvement of the patients suffering from atherothrombotic diseases.

This is only a preliminary study and to make final comment the extracts should thoroughly investigate phytochemical and pharmacological to exploit their medical and pharmaceutical potentialities.

Finally, green tea leaf extract of different solvents in this research exhibited different degrees of antioxidant activity. This study indicates that green tea is one of the most effective plant in terms of antioxidant properties and can serve as natural sources to the free radical scavengers and antioxidant agents. Green tea leaves *can* be considered as promising sources of natural antioxidants and as possible preventative agents of some common human health disorders. However, the total phenolic and flavonoid content showed a weak correlation with the antioxidant activity of the investigated plant. Hence, detailed studies on the role of individual phytochemicals involved in the antioxidant activity of this plant are required for its use as functional food, beverage and in the pharmaceutical industry.

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**Table 1.** *In vitro* antibacterial activity of water, ethanol and acetone extracts of leaves of *Camellia sinensis*

Test Bacteria	Zone of inhibition in diameter (mm)			
	Water extract (0.5mg/disc)	Ethanol extract (0.5mg/disc)	Acetone extract (0.5mg/disc)	Standard (0.1mg/disc)
<i>Vibrio cholerae</i>	11.3	NS	11.0	14.0
<i>Staphylococcus aureus</i>	12.0	11.5	10.0	16.5
<i>Salmonella typhi</i>	11.6	10.8	9.5	20.0
<i>Pseudomonas aeruginosa</i>	NS	9.5	NS	25.5
<i>Bacillus subtilis</i>	13.0	12.7	8.0	30.0
<i>Bacillus cereus</i>	NS	NS	10.2	18.2
<i>Shigella dysenteriae</i>	13.0	11.6	10.0	34.5
<i>Staphylococcus pyogenus</i>	NS	9.9	7.5	31.0
<i>Escherichia coli</i>	14.2	11.0	9.0	29.5
<i>Bacillus megaterium</i>	10.5	NS	NS	28.0

Standard (Amoxicillin solution)

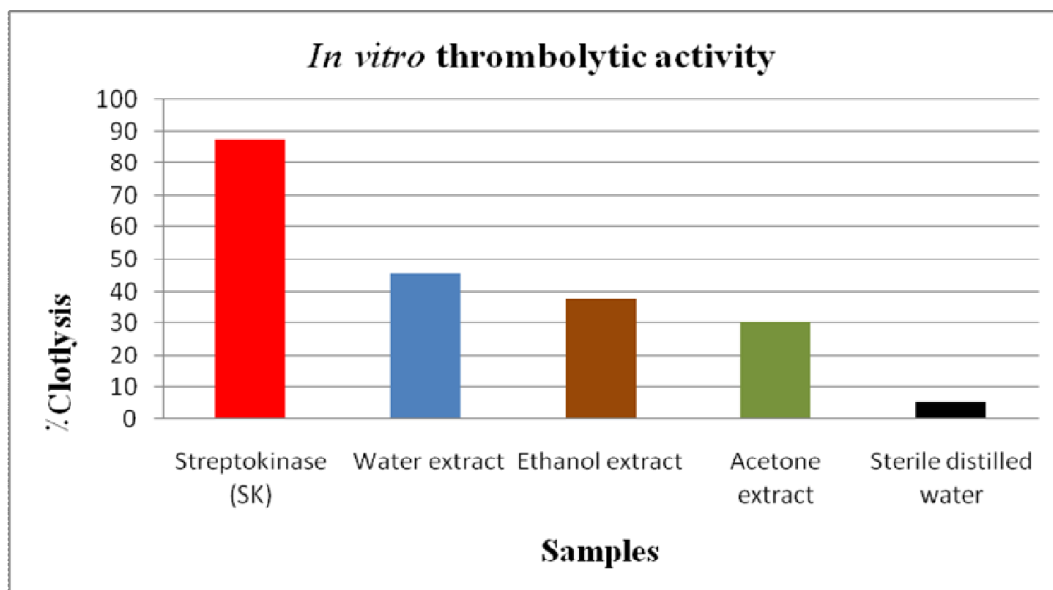
NS = Not Susceptible

**Table 2.** Effect of different solvent extracts of green tea leaves on *in vitro* thrombolysis

Fractions/Drug	% Clot lysis (mean $\pm$ S.D)	P value (Two-tailed) when compared to negative control (water)
Streptokinase (SK)	87.15 $\pm$ 3.212	< 0.0001
Water extract	45.60 $\pm$ 2.313	< 0.0001
Ethanol extract	37.68 $\pm$ 2.211	< 0.0001
Acetone extract	30.51 $\pm$ 2.551	< 0.0001
Sterile distilled water	5.60 $\pm$ 2.131	< 0.0001

**Table 3.** IC<sub>50</sub> values of DPPH scavenging activity, total flavonoid content (TFC) and total phenolic content (TPC) of three different solvent extracts of green tea leaves

Samples	% inhibition of DPPH	TFC ( $\mu$ g /mg)	Total phenols (mgGAE/g extract)
Water extracts	88.32 $\pm$ 1.53	78.16 $\pm$ 1.90	160.00 $\pm$ 0.62
Ethanol extracts	78.80 $\pm$ 1.73	65.99 $\pm$ 4.11	123.33 $\pm$ 2.32
Acetone extracts	61.96 $\pm$ 1.53	55.85 $\pm$ 1.43	95.37 $\pm$ 1.12



**Figure 1.** Thrombolysis by streptokinase (SK), various solvent extracts of green tea leaves, and distilled water (as a negative control)