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# COMPARATIVE STUDIES ON ANTIOXIDANT AND THROMBOLYTIC ACTIVITIES OF METHANOL AND ETHYLACETATE EXTRACTS OF TREMA ORIENTALIS

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## ABSTRACT

The present study was carried out to evaluate in-vitro antioxidant, and thrombolytic activity of MeOH and EtOAc extracts Trema orientalis flowers. Antioxidant activity of the extracts was tested by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay method whereas the thrombolytic activity was tested by in-vitro clot lysis assay method. The MeOH extract of T. orientalis showed mild free radical scavenging activity ($IC_{50}$: 131.2µg/ml) compared to ascorbic acid ($IC_{50}$:12.4µg/ml) used as a standard. On the other hand, both MeOH and EtOAc extract produced 21.4 and 21.5% clot lysis respectively compared to streptokinase used as a positive control that caused 51% clot lysis.

**Keyword:** DPPH, streptokinase, antioxidant activity, thrombolytic activity, Trema orientalis

## INTRODUCTION

A free radical is a highly reactive atom, molecule or ion having unpaired valence electrons or an open electron shell. Excessive amounts of these free radicals in human body can lead to cell injury and death. Imbalance between ROS (Reactive Oxygen Species) and a biological system's ability to detoxify the reactive free radical intermediate is termed as oxidative stress. In human body oxidative stress can cause cancer,[1] atherosclerosis, heart failure,[2] autism,[3] Parkinson's disease, Alzheimer's disease,[4][5]and sickle cell disease.[6] Antioxidant is a molecule which inhibits the oxidation of other molecules through terminating the chain reactions by removing free radical intermediates. Various parts of plants like barks, leaves, roots, flowers, seeds, fruits, etc. as well as full plant is considered as a rich source of antioxidants such as vitamin C, vitamin A, vitamin E etc.[7][8]

Thrombus or blood clot may cause blockage of blood flow in a small blood vessel which leads the risk of cardiac arrhythmia, myocardial infarction and stroke.[9] Breakdown or lysis of blood clot is termed as thrombolysis [10] is caused by tissue plasminogen activator (tPA).Intravenous heparin is used as the first-line therapy because of its safety profile and activity.[11] Many drugs have been developed with the development of modern pharmaceutical science such as anistreplase, alteplase, urokinase, streptokinase and tissue plasminogen (TPA), etc.[12][13]Some medicinal plants are also reported to show thrombolytic activity.[14][15]As part of our continuous search for medicinal plants with thrombolytic activity, we selected *T. orientalis* flowers. *T.orientalis* is a species of flowering tree from Cannabaceae family and distributed in tropical and warm temperate zones. The tree has various uses as an herbal medicine; leaves and the barks are widely used to treat coughs, sore throats, asthma, bronchitis, gonorrhoea, dysentery, yellow fever, toothache, as well as an antidote to general poisoning.[16][17] According to recent pharmacological studies, an aqueous extract of the bark has been shown the property to reduce blood sugar levels in an experimental animal model of diabetes mellitus and can be useful for treating this disease.[18]

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Here, we report the antioxidant and thrombolytic activity of T. orientalis flowers.

## MATERIALS AND METHOD

### Chemicals

The standard sample Streptokinase was purchased from the local market Dhaka, manufactured by BEACON pharmaceuticals Ltd, Bangladesh.

### Plant material

The flowers of *Trema orientalis* were collected from the district of Jessore, Bangladesh in January 2015, and were identified by the experts at Bangladesh National Herbarium, Dhaka, Bangladesh.

### Preparation of the crude extract

The flowers were shed dried. The dried flowers were powdered with the help of mechanical grinder after overnight drying in an oven below 50°C. At room temperature about 435 g of powder was taken in a clean, flat-bottomed glass container and soaked in 1800 ml of 90% methanol and 300 g of powder soaked in 1.1 L of EtOAC. The container with its contents was sealed and kept for a period of 10 days accompanying occasional stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material for several times. Then it was filtered through Whatman filter paper.

### Phytochemical screening

Phytochemical screening of Trema orientalis was carried out to identify the functional groups as described. [19-21]

### Antioxidant test

Antioxidant activity of the extracts was determined on the basis of their scavenging potential of the stable DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) free radical in a quantitative assay. [22-24]In short, 2.5 mg extract was mixed with 25 mL of ethanol to prepare 100 µg/mL solution of the extract as stock solution. From the stock solution of the extract, a serial dilution was carried out to obtain a concentration of 1, 5, 10, 50, 100, 500 µg/ml. Test tubes and volumetric flasks are wrapped with foil paper. In 6 Test tubes, serial dilution of the extract is done and marked them respectively. 1ml of the sample from each concentration and 3 ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. Then the solution is kept in the dark place for 30 minutes with raping each test tube with foil paper. In another test tube 3ml 0.004% DPPH & 1ml MeOH was taken to prepare a blank solution. Then absorbance is taken at 517nm by UV Spectroscopy.

**Table 1 : Chemical group tests of T. orientalis**

| Tested groups | EtOAcextract | MeOHextract |
|---|---|---|
| **Saponin** | - | + |
| **Glycoside** | + | + |
| **Flavonoids** | - | + |
| **Tannin** | - | + |
| **Alkaloids** | + | + |
| **Steroid** | - | - |

**(+) Indicates presence, (–) Indicates absence.**

**Table 2 : $IC_{50}$ values of the extracts of T.orientalis**

| Test Samples | $IC_{50}$ value (µg/ml) |
|---|---|
| Ascorbic Acid | 12.39 |
| MeOH extract | 131.367 |
| EtOAC extract | >500 |

### Thrombolytic activity Test

Thrombolytic test was carried out by percentage of clot lysis method.[25] In short, blood was drawn from healthy volunteers (n=3) without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of venous blood was transferred to the each pre-weighed microcentrifuge tubes and incubated at 37° C for 45 min and was allowed to clot. The thrombolytic activity of all extracts was evaluated using streptokinase (SK) as the standard substance. The extractive (100 mg) from each plant was suspended in 10 ml of distilled water and was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22 micron syringe filter. After clot formation, the serum was completely removed without disturbing the clot and each tube containing the clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube with the pre-weighed clot, 100 µl aqueous solution of crude extract was added separately. Then, 100 µl of streptokinase (30,000 IU) and 100 µl of distilled water were separately added to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 min and observed for lysis of the clot. After incubation, the released fluid was removed, and tubes were again

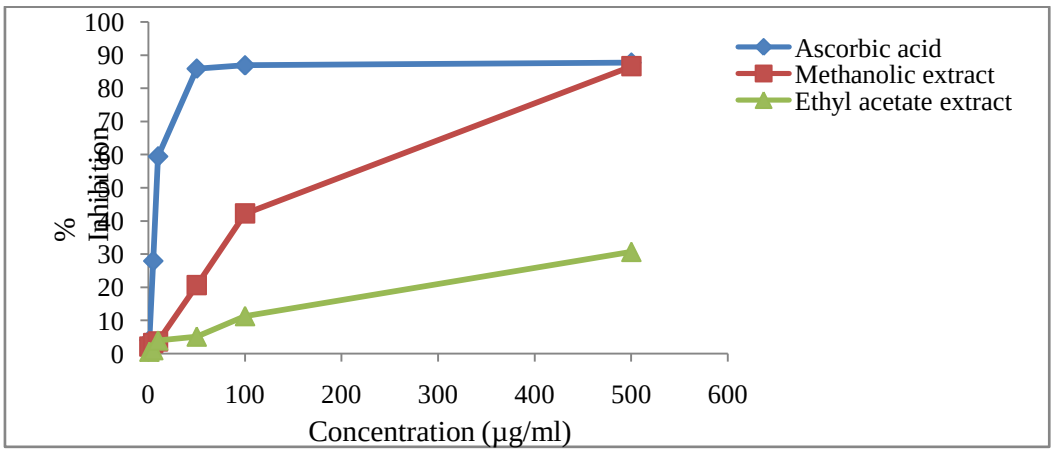


Fig. 1 :

**Table 3 : Thrombolytic activity test**

| Sample | Wt. of Blank tube (g) | 1st clot + tube (g) | 1st clot (g) | 2nd clot + tube (g) | 2nd clot (g) | Lysis weight (g) | % of lysis |
|---|---|---|---|---|---|---|---|
| SK | 0.84±0.01 | 1.76± 0.01 | 0.92±0.00 | 1.29±0.02 | 0.45±0.00 | 0.47±0.00 | 51.08 |
| DW | 0.82±0.00 | 1.47±0.06 | 0.65±0.06 | 1.44±0.04 | 0.61±0.03 | 0.04±0.02 | 6.15 |
| TOMEx | 0.84±0.01 | 1.54±0.01 | 0.70±0.00 | 1.39±0.04 | 0.55±0.02 | 0.15±0.02 | 21.43 |
| TOEAEx | 0.85±0.02 | 1.50±0.15 | 0.65±0.16 | 1.36±0.06 | 0.51±0.08 | 0.14±0.09 | 21.54 |

**SK=Streptokinase as a standard reference, DW=Distill Water as control, TOMEx=*T.orientalis* MeOH extract, TOEAEx= *T.orientalis* EtOAc extract. Values are represented as Mean±SD.**

weighed to observe the difference in weight after clot disruption.

## RESULTS AND DISCUSSION

### Phytochemical Screening

Results of the phytochemical screening of the MeOH and EtOAc extract of *Trema orientalis* (Flowers) Table-1 showed the results of the phytochemical screening of the MeOH and EtOAc extract of *T. orientalis.* Results indicated the presence of saponin, glycoside, flavonoids, tannins, and alkaloids in MeOH extract, whereas in EtOAc extract glycoside and alkaloid were present.

### Thrombolytic activity

Thrombolytic test was carried out by percentage of clot lysis method. As per discovery of new clot lysis drugs from the natural sources, *T. orientalis* extracts were assessed. Table-3 represents the results in which 100 µl SK, a positive control (30,000 I.U.), was used for comparison.

MeOH and EtOAc extract of *T. orientalis* showed 21.4% and 21.5% clot lysis, respectively, compared to control. Streptokinase, used as the reference standard, showed 51.1 % clot lysis (Table-3). From the results it can be suggested that the both MeOH and ethyl acetate extract of *T. orientalis* had a moderate thrombolytic activity.

## DISCUSSION

Plants already recognized as a medicinal plant but still many plants are not explored and used which have various medicinal values. Plants contain many novel compounds which play a vital role in the treatment of different diseases such as Tannins show anti-inflammatory and anticancer activity [26][27] Flavonoids act as antioxidant, anti-inflammatory and anticancer agents [28] Alkaloids also possess antileukemic and anticancer activity [29] and Saponin is antimicrobial agent and maintains the blood cholesterol level [30]. A large number of plants source have been studied for their supplements having anticoagulant and anti-platelet activity which prevent the risk of coronary events and stroke [31][32]. *T. orientalis* extract contains Saponin, Glycoside, Flavonoids, Tannin, and Alkaloids. From table-3 it was found that the MeOH extract showed mild scavenging activity compared to ascorbic acid, it may be correlated to the presence of flavonoids.

## CONCLUSION

The present study revealed that extracts of the *T. orientalis* could be used as a source for cardio and cerebral protective and antioxidant activity. At last, we can say that further study is needed to do in-vivo antioxidant activity and thrombolytic activity and find out the causative metabolites of *T. orientalis* and possible mechanism of antioxidant and clot lysis activities.

### Competing Interests

The authors declare that they have no Competing Interests.

### Authors Contributions

AKA, SL and IUR prepared extracts and carried out experiments. FA closely supervised throughout the works. Manuscript writing, data analysis, thoroughly reading and final approval was the team efforts of OI, SA, MK, MI, CS, ND, NAS, SH, SPH, MR,KB and AI.

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