

Thrombolytic and Anti-arthritic Activities of Methanolic Extract of *Trevesia palmata*

Mohammed Aktar Sayeed¹, Md. Faruk², Ahmad Ibtehaz Chowdhury², Ahmmed Rusti Faisal^{2*}, Abdullah Al Mamun², Mujahidul Islam²

1. Associate Professor, Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh.
2. Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh.

*Corresponding Author: Ahmmed Rusti Faisal, C/O - Mohammed Aktar Sayeed, Associate Professor, Department of Pharmacy, International Islamic University Chittagong, 109, Chatteswary Road, Chawkbazar, Chittagong-4203, Bangladesh.
e-mail: pharmfaisal5@gmail.com Contact no. : +8801820938261

Abstract

The crude methanol extract of the leaves of *Trevesia palmata* was partitioned with n-hexane, ethyl acetate, chloroform and water for biological investigation. Methanol extract of *Trevesia palmata* was assessed for *in vitro* thrombolytic and anti-arthritic activity. Anti-arthritic activity was evaluated using albumin denaturation. Diclofenac sodium (D.s.) was used as a standard drug for the study of anti-inflammatory activity. The methanol extract of *Trevesia palmata* showed mean inhibition of protein denaturation 89.24 ± 0.6584 at $1000 \mu\text{g/ml}$ whereas, standard diclofenac sodium it was found to be 96.77 ± 1.1404 . In thrombolytic activity using *in vitro* clot lysis assay method, the ethyl acetate soluble fraction (LEAF) and n-hexane soluble fraction (LHXF) of leaf extract was found to have significant ($p < 0.001$), thrombolytic activity showed a maximum effect of 44.293 ± 0.5069 & 44.211 ± 0.7428 while the standard streptokinase showed 71.668 ± 0.466 .

Key words: *Trevesia palmata*, thrombolytic, anti arthritic, clot lysis, inhibition, protein denaturation.

Introduction

Trevesia palmata is a plant species of the family *Araliaceae*. It is native to Asia: China, Bangladesh, Bhutan, India, Nepal, Cambodia, Laos, Myanmar, Thailand and Vietnam. This species is used medicinally and as an ornamental plant.

A blood clot (thrombus) developed in the circulatory system due to the failure of homeostasis causes vascular blockage and leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death. Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots^[1]. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs^[2]. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Selective thrombin inhibitors and anti platelet agents are

more potent, but their safety remains to be confirmed. Several third generation thrombolytic agents have been developed. Compared to the second generation agents (alteplase), the third generation thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase result in a greater angiographic potency in patients with acute myocardial infarction, although, so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials. Bleeding risk, however, may be greater ^[3]. Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase is an example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA ^[4]. Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often perceived as safe because they are "natural" ^[5]. Epidemiologic studies have provided evidence that foods with experimentally proven anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported ^[6].

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage ^[7]. It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female ^[8]. Its prevalence depends upon age ^[9]. Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity ^[10]. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas ^[11]. Number of synthetic medicines has been derived from medicinal herbs ^[12]. The sapodilla is large, evergreen, forest tree more than 30 m in height and with a diameter up to 1.5 m, under cultivation it varies between 9 and 15 m, depending on location, and generally does not exceed 50 cm in diameter. The gummy latex of sapodilla, called chicle, is used to make chewing gums, and the fruit is used to treat diarrhea and pulmonary diseases ^[13]. The crushed seeds are claimed to expel bladder and kidney stones and effective in rheumatism ^[14]. Leaf decoction used for fever, hemorrhage, wounds and ulcers ^[15].

The aim of our present work was to investigate the selected methanolic extracts of *Trevesia palmata* for probable thrombolytic and anti arthritic activities by using an *in vitro* procedure ^[16] and we, here in, report the results of our preliminary investigations.

Materials and Methods

Plant materials

Adequate amount fresh leaves of *Trevesia palmata* for this study were collected from the hill tracts of Sitakunda area, Chittagong, Bangladesh and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh.

Extraction

The leaves were dried at room temperature in shade for 5 days and in hot air oven for 2 days. The dried leaves were coarsely powdered and extracted with methanol for 7 days. The sediments were filtered and the filtrates were evaporated to dryness at 40°C. The dried crude extract (14 gm) was then partitioned successively with n-Hexane, chloroform, and ethyl acetate by using modified Kupchan partitioning method. The

resultant partitionates i.e. n-hexane (LHXF),) chloroform (LCHF), ethyl acetate (LEAF) and aqueous (LAQF) soluble fractions were stored at 4°C until used for experimental process. The solvent was completely removed and the dried extract was used for the experiment.

Thrombolytic activity

In vitro clot lysis study:

Streptokinase (SK): About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15,00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolytic^[16] study.

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^[16].

Clot lysis: Experiments for clot lysis were carried as reported earlier^[16]. Venous blood drawn from healthy volunteers ($n = 10$) was 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 tube alone). Each alpine tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes. 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 test was repeated ten times.

Statistical analysis: The significance between % clot lysis by herbal extract by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean ± standard deviation.

In vitro anti-arthritic activity by protein denaturation inhibition method^[17]:

The solutions used were the test solution (0.5ml) consisting of plant extract of 0.05ml (250µg/ml) and bovine serum albumin of 0.45ml (5% w/v aqueous solution); the test control solution (0.5ml) consisting of 0.45ml bovine serum albumin and 0.05ml of distilled water; the product control solution (0.5ml) consisting of 0.45ml of distilled water and 0.05ml of plant extract and the standard solution (0.5ml) consisting of 0.45ml of Bovine serum albumin and 0.05ml of Diclofenac sodium (250µg/ml). Then pH was adjusted to 6.3 using 1N HCl and incubated at 37°C for 20 min and later at 57°C for 3min. After cooling the solutions, 2.5ml of phosphate buffer was added, and then the absorbance was measured at 416nm. The percentage inhibition of protein denaturation was calculated as

$$\% \text{ inhibition} = \frac{100 - \{(\text{Absorbance of test solution} - \text{Absorbance of product control}) \times 100\}}{\text{Absorbance of test control}}$$

Statistical Analysis

Data are presented as the mean \pm SEM of each triplicate test. The analysis was performed by using Dunnett vs. Control test and by ANOVA. $P < 0.001$ were considered to be statistically significant.

Results and Discussion

Antiarthritic activity

Table 1: Anti arthritic activity in *Trevesia palmata* leaf extract

| observation | Absorbance | % of inhibition \pm S.E.M. |
|---|---------------------|------------------------------|
| METP (1000 μ g/ml) | 0.263 \pm 0.0004 | 89.24 \pm 0.6584** |
| Positive control D.S. (1000 μ g/ml) | 0.258 \pm 0.00082 | 96.77 \pm 1.1404 |

**p < 0.01, significant compared to control.

In the present study for *in-vitro* anti-arthritic test, the 1000 mg/kg of crude methanol extract of *Trevesia palmata* showed mean inhibition of protein denaturation 89.24 \pm 0.6584% and whereas for standard diclofenac sodium(D.S), it was found to be 96.77 \pm 1.1404%.

Table 1. Effect of *Trevesia palmata* extract and fraction on *in vitro* clot lysis

| Sample | % of thrombolysis \pm SEM | P-Value |
|---------------------------------|-----------------------------|---------|
| Streptokinase(positive control) | 71.668 \pm 0.4661 | <0.001 |
| Water(negative control) | 5.54 \pm 0.9555 | <0.001 |
| METP | 43.298 \pm 0.8647*** | <0.001 |
| LHXF | 44.211 \pm 0.7428*** | <0.001 |
| LCHF | 43.280 \pm 0.8724*** | <0.001 |
| LAQF | 40.332 \pm 0.2913*** | <0.001 |
| LEAF | 44.293 \pm 0.5069*** | <0.001 |

***p < 0.001, significant compared to control.

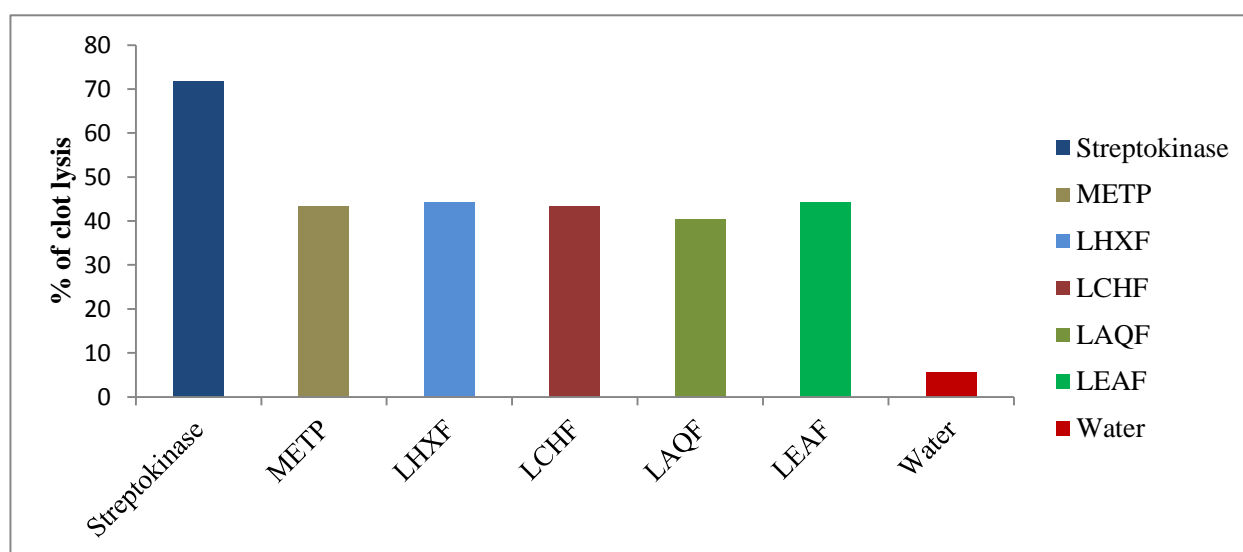


Figure 1. The clot lysis activity of extract of *Trevesia palmata*, Streptokinase, and Water.

Thrombolytic activity

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37 °C, showed 71.668±0.4661 % clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (5.54±0.9555%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.001). Fraction of *Trevesia palmata* LEAF show maximum 44.293±0.5069% significant thrombolytic activity then other show METP 43.298±0.8647%, LHXF 44.211±0.7428%, LCHF 43.280±0.8724%, LAQF 40.332±0.2913% clot lysis.

Discussion

The result of this work showed that the extract of *Trevesia palmata* had significant anti arthritic activity (Table 1). A huge numbers of thrombolytic drugs are obtained from different sources and then further modified, using recombinant technology. This is performed to make them more site-specific and to reduce their side-effect. Sometime patient may dies due to bleeding or internal hemorrhage, so direct use of herbal thrombolytic drug is not expected. Their further modification is required. The observed thrombolytic percentage (table-2, fig-1) shows quite good effect of thrombolysis of the extract and fraction of *Trevesia palmata* leaves. It possesses a significant percent of thrombolysis comparing with streptokinase (positive control) and makes a difference from negative control (water). So it can be concluded as extract has significant anti arthritic activity and significant thrombolytic agent.

Conclusion

Since the extract and fraction of *Trevesia palmata* leaves shows significant thrombolytic and anti arthritic properties, the further laboratory study and chemical isolation of this plant might confirm an effective drug in pharmacologic aspects as anti-coagulant and in anti-inflammatory therapy. Methanolic extract of *Trevesia palmata* has most promising data in both assays that suggest its effectivity in both types of pharmaceutical arena.

References

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