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Trigonella foenum-graecum sprouted seed aqueous extract exhibits strong anticoagulant and fibrinogenolytic activities: Role of serine protease

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Abstract
Trigonella foenum-graecum sprouted seed aqueous extract (TFS extract) exhibited fibrinogenolytic, anti-coagulant (both *in vitro* and *in vivo*) and anti-platelet aggregating activities. TFS extract hydrolysed casein with a specific activity of 0.6 U. TFS showed increased activity in terms of clotting time of citrated plasma by 2.04 and 1.61 folds in RT and PT respectively. TFS extract inhibited ADP induced platelet aggregation to an extent of 65%. Further, the *in vivo* studies showed the prolonged tail bleeding time in dose dependent study using mouse model, which supported anticoagulant property. The TFS specifically prolonged the clotting time in PT (from 19±1 sec to 30±0.58 sec) and RT (from 3.6±0.3 min to 7.44±0.09 min) revealing the involvement of extract in extrinsic pathway of coagulation. TFS extract completely hydrolyzed all the chains Aα, Bβ and γ chains of fibrinogen. The proteolytic activity of TFS extract was completely inhibited by PMSF, however E- 64, EDTA and pepstatin-A did not, indicating the presence of serine type of protease. TFS extract was devoid of hemolytic, edema inducing and hemorrhagic activities suggesting its non-toxic nature. TFS has significant anticoagulant bioactive molecule/s which can be useful for further thrombotic disorder treatment.

Keywords: *Trigonella foenum-graecum* sprouted seed aqueous extract; serine protease; anticoagulant; fibrinogenolytic activity; anti-platelet aggregation; tail bleeding assay; non-toxic

Introduction
Hemostasis is a complex cascade of reaction highly regulated by series of proteins. The zymogen form of the proteins gets converted into active proteases by hydrolysis process. There are two pathways for the initiation of the cascade: the intrinsic pathway triggered by negatively charged foreign particles while the extrinsic pathway by blood vessel wall exposure. Both the pathways merge at the activation of factor X. The activated factor Xa complexes with factor V with phospholipids of membrane surface and Ca^{+2}. This complex converts prothrombin to thrombin, a potent serine protease. Thrombin acts on solable fibrinogen converting it to insoluable fibrin, this process stabilized by factor XIII which in turn stops bleeding [1, 2].
Several hemostatic agents have been reported from different sources such as venoms, microbes and plant latex [3-6]. Medicinal plant extracts have been extensively used as hemostatic agent in folk/ayurvedic medicinal treatment.
Trigonella foenum-graecum belongs to family Fabaceae is native to Eastern Europe while widely cultivated in all parts of the world. It is an erect annual herb, the leaves are used as vegetable and dried leaves used as food additives in India. The whole plant has also been used to treat diseases susch as bronchitis, fever, sore throat, wound, swollen glands, skin irritation, ulcers and diabetes. The seeds are aromatic, bitter in taste, carminative and antibacterial in nature. The seeds extensively used in ayurvedic medicinal treatment as carminative, expectorant, laxative an stomachic agent [7]. The mature seeds were reported to contain aminoacids, fatty acids, vitamins, saponins, flavonoids, polysaccharides and alkaloids [8-10]. Newer researchers have identified hypocholesterolemic, antilipidemia, antioxidant, hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiulcer, antilithigenic and anticarcinogenic effects [11].
In spite of its medicinal value the *T. foenum-graecum* has not been explored for its effect on blood coagulation. Thus the current study evaluates the anticoagulant, antiplatelet aggregating and fibrinogenolytic activities of TFS extract.

Materials and Methods
Materials
Casein, gelatin (from porcine skin), fibrinogen (from human plasma), E-64, phenyl methyl sulphonyl fluoride (PMSF), ethylenediaminetetraacetic acid (EDTA), and pepstatin A from Sigma Aldrich (St. Louis, MO, USA). Trypsin from HiMedia (Mumbai, Maharashtra, India), and all other reagents used were of analytical grade. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

Animals
Swiss albino mice weighing 20-25 g were used in the study. All the experimental procedures, animal handling and sampling were conducted according to the guidelines of institutional animal ethical committee. Mice were maintained in polypylene cages under controlled conditions (28 ºC; humidity 45%) with 12 h day-light cycle until acclimatization. Animals were fed with standard mice diet and water ad libitum during the experimental period. (All animal care and experimental procedures complied with the guidelines of the institutional animal ethics committee constituted by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Registration no. 123/PO/C/99/CPCSEA; sanction letter no. SSCPT/IAEC. clear/152A/2016–17 dated October 01, 2016.

Trigonella foenum-graecum sprouted seed aqueous extract preparation

T. foenum-graecum seeds were purchased from local market. Seeds were soaked overnight in distilled water. The soaked seeds are kept in closed container for sprouting. After two days the sprouted seeds were shade dried. The dried sprouted seeds were finely powdered and stored.

The powder (10 g) was suspended in distilled water (100 ml) and kept on magnetic stirrer overnight at a speed of 500 rpm for extraction. Later the extract was centrifuged; supernatant is concentrated and used for further studies. The extract was named as TFS extract.

Proteolytic activity

Proteolytic activity was analysed as explained in Satake *et al*. [12]. The casein (2%) dissolved in 200 mM Tris-HCl buffer (pH 7.0) was used as substrate. Various concentrations of TFS extract (0–200 µg/20 µl) along with standard trypsin were incubated with 0.4 ml of casein separately at temperature 37ºC for 2 hours 30 min. This reaction was terminated using 1.5 ml of trichloroacetic acid (0.44 M). After 30 min, centrifugation was done with the speed 3,000 rpm for 5 min. To the 1 ml of supernatant 0.4 M sodium carbonate (2.5 ml) and 1:2 diluted Folin–Ciocalteu (FC) (0.5 ml) reagents were added and incubated at 37ºC for 20 min. The developed colour was analysed at 660 nm (Shimadzu UV-Vis spectrophotometer). One unit of enzyme activity is stated as the quantity of enzyme required to increase the absorbance by 0.01 at 660 nm in 1 h. To know the type of protease the TFS extract was incubated with 5 mM concentrations of standard protease inhibitors (E-64, EDTA, PMSF, pepstatin A).

Sodium Dodecyl Sulphate –Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed as explained in Laemmli [13]. TFS extract (80 µg) was treated with a non-reducing and reducing sample buffer and kept in boiling water bath for 3 min. SDS-PAGE (10%) was performed for TFS extract and molecular weight standards ranging between 14.2 - 97.0 kDa. The protein bands obtained were detected using 0.25% Coomassie brilliant blue R- 250 (CBBR-250).

Periodic acid–Schiff base (PAS) staining

PAS staining was performed, as explained in Leach *et al*. [14]. SDS-PAGE was performed for 80 µg of TFS extract and 50 µg of fibrinogen (+ve control). The gel was fixed in 7.5% acetic acid temperature 37 ºC for one hour, and then washed with nitric acid. Further, the gel was kept at 4℃ in aqueous periodic acid (0.2%) for 45 min. This gel was stored overnight at 4 ºC in the Schiff reagent. To visualize the reddish-pink bands the gel was de-stained with 10% acetic acid.

Zymogram assay

Zymography was performed as according to the method of Laemmli [13]. The 10% SDS-PAGE was performed for 80 µg of TFS extract, where the resolving gel was incorporated with 0.2% casein/gelatin separately. After electrophoresis, the SDS was removed by washing the gel using Triton X-100 (2.5%). Further, the gel was incubated in Tris–HCl (50 mM; pH 8.0) buffer containing $CaCl_2$ (10 mM), and NaCl (150 mM). The gel was stained using CBBR-250 (0.25%) to observe activity bands.

Fibrinogenolytic activity

The fibrinogenolytic activity was evaluated as explained in Nagaraju and Kemparaju. [6]. 50 µg of human fibrinogen was treated with various concentrations (0–15 µg/20 µl) of TFS extract at 37 ºC for 2 hours 30 min. Reducing sample buffer (10) was used to terminate the reaction. The hydrolysed products were analysed in SDS-PAGE (12%) and stained with 0.25% of CBBR-250 to visualize bands.

Coagulation studies

Recalcification time (RT)

Recalcification time assay was carried out as explained in Condrea *et al*. [15]. Different concentration (50 - 150 µg/20 µl) of TFS extract was treated with 100 µl of citrated human plasma at 37 ºC for 5 min. Further, 25 mM $CaCl_2$ (100 µl) was added and recorded the time until the formation of a clot.

Prothrombin time (PT)

PT was determined according to Quick *et al*. [16]. Citrated human plasma (100 µl) was incubated with different concentration of TFS extract (50 - 250 µg/20 µl) at 37 ºC for 15 min. After incubation, 0.2 ml of liquid thromboplastin (Uniplastin) was added and time taken for clot formation was recorded against a light source.

Platelet aggregation

Platelet aggregation was analysed in Chrono-Log Model 700-2D aggregometer (ChronoLog Corp., USA). We collected blood from healthy volunteers. Platelet-rich plasma (PRP) was obtained by centrifuging human blood (1:9 v/v in 3.2% trisodium citrate) at 900 rpm for 15 minutes; the resultant supernatant was PRP. The blood sample was centrifuged at 3,000 rpm for 20 min to obtain Platelet Poor Plasma (PPP) as the supernatant. 250 µl of PRP was taken in siliconized glass cuvette with stir bar stirring at 1200 rpm. The platelet aggregation was initiated by adding ADP (10 µM). Different concentration of TFS extract (50, 100, 150 and 200 µg) incubated with PRP for 15 minutes and aggregation were initiated by adding ADP. The aggregation was monitored for 6 min by change in turbidity, with PRP and PPP representing 0% and 100% transmittance, respectively.

Tail bleeding assay

The tail bleeding time assay was done according to the method of Denis *et al*. [17]. Briefly, TFS extract (5, 10 and 15 mg/kg body weight) in 50 µl of saline was injected intravenously through the tail vein of a group of six mice. After 10 min the mice were anesthetized using diethyl ether and a sharp cut of 3 mm length at the tip of the mouse was made. Immediately, the tail was vertically immersed into saline, which was prewarmed at 37º C. The bleeding time was recorded until the bleeding stops. For inhibition studies, TFS extract was preincubated with 5 mM PMSF for 15 min at 37º C.

Hemolytic activity

The assay was carried out as explained by Shin *et al*. [18]. Blood from a healthy human individual was treated with 3.2% of trisodium citrate in the ratio 1:9 and centrifuged for 5 min at speed 3,000 rpm to obtain red blood cell (RBC) pellet. The pellet was washed and re-suspended in normal saline (0.9% NaCl) to obtain 2% erythrocyte suspension.

The MzNL with the concentration ranging from 0-500 µg/50 µl were treated with 0.5 ml erythrocyte suspension in a reaction volume of 1 ml made with saline and incubated at temperature 37ºC for 30 min. Further, normal saline (2 ml) was pipette to the mixture and this mixture was subjected to centrifugation at speed 1,500 rpm for 2–3 min. Distilled water was used to obtain maximal hemolytic control.

Hemorrhagic activity

Hemorrhagic activity was done according to the method of Kondo *et al*. [19]. Different concentrations of TFS extract (0 – 200 µg) was injected independently through intradermal route to groups of five mice each in 50 µl of saline. Group of mice received saline alone serves as negative control while group received *Calotropis gigantia* latex (2 MHD) as positive control. After 3 h, mice were sacrificed and dorsal patch of skin was observed for hemorrhage against saline injected control mice. The diameter of the hemorrhagic spot was measured. The minimum hemorrhagic dose (MHD) was defined as the amount of the protein producing 10 mm of hemorrhage in diameter.

Edema inducing activity

Edema inducing activity was done according to the method of Vishwanath *et al*. [20]. Different concentration of TFS extract (0-200 µg in 20 µl) was injected to group of mice into the right foot pads. The left foot pads were injected with saline alone which serves as negative control. After 1 h mice were sacrificed and hind limbs were removed at the ankle joint and weighed. Increase in weight was calculated as edema ratio, which equals the weight of edematous leg x 100/weight of normal leg. Minimum Edema Dose (MED) was defined as the amount of protein required to cause an edema of 120%.

Protein estimation

The protein estimation was performed in accordance with Lowry *et al*. [21]. Protein measurement with the folin phenol reagent, where Bovine serum albumin (BSA) was used as standard**.**

Statistical analysis

Results were represented as mean ± standard error. One-way analysis of variance was used for statistical significance of intergroup differences, and the Tukey test was used for comparison of means. The Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA) was used to perform all analysis.

Results

3.1. Electrophoresis and zymography:

The protein banding pattern and the nature of proteins of TFS was identified by SDS-PAGE and PAS staining. TFS extract was subjected to 10% SDS-PAGE under non-reducing and reduced conditions. TFS extract showed bands distributed majorly in medium molecular weight region and in low molecular weight region. In reduced condition, majority of the low molecular weight region proteins reduced as observed by the low intensity in the band (Fig. 1). Upon analysis for the presence of glycosylated proteins by PAS staining, TFS extract showed glycoprotein bands in medium and low molecular weight regions (pink color bands in Fig. 2).

Proteolytic activity

The protease activity of TFS extract was assayed by using denatured casein as substrate. Trypsin was used as the standard for comparing the protease activity of TFS extract. TFS extract showed a specific activity of 0.8 U, while trypsin exhibited a specific activity of 6.1 U. The results of the gelatin zymography, which demonstrated transluscent activity bands in high molecular weight region (Fig. 3) provide the evidence for supporting the protelytic activity in TFS extract. To evaluate the type of protease present in TFS extract, the extract was incubated with standard protease inhibitors such as E-64, PMSF, EDTA and pepstatin A. The proteolytic activity was strongly inhibited by PMSF (90%) which indicate that TFS extract contains a serine-type of protease (Table. 1).

Fibrinogenolytic activity

The pharmacological activity of TFS extract was studied by using human fibrinogen. Fibrinogen is a 340 kDa soluble plasma fibrinogen glycoprotein composed of three subunits named as Aα, Bβ and γ. It plays a major role in arresting blood flow during tissue/vascular injury by converting to insoluble fibrin and subsequently fibrin-based blood clot.

To study the fibrinogenolytic activity of TFS extract, fibrinogen incubated with TFS extract analysed by dose dependent and time dependent degradation pattern. In dose dependent effect TFS extract at the concentration of 30 µg degraded the Aα chain while Bβ and γ chains of are resistant (Fig. 4). In time dependent assay, fibrinogen was incubated with TFS at a concentration of 50 µg for different time interval and TFS at 24 h degraded all the bands of fibrinogen (Fig. 5a). The effect of standard protease inhibitor was analyzed for fibrinogenolytic activity of TFS extract. The fibrinogenolytic activity of TFS extract was completely inhibited by PMSF suggesting the serine type of protease involved in fibrinogenolytic activity (Fig. 5b)

Coagulation assays

The anticoagulant potential of TFS extract was evaluated by recalcification time and prothrombin time assays. TFS extract exhibited strong anticoagulant activity by interfering in plasma coagulation cascade. It enhanced the clotting time of plasma from 3 min 06 sec to 7 min 44 sec in recalcification time assay at a concentration of 200 µg (Fig. 6). TFS extract increased the clotting of plasma from 19 sec to 30 sec in prothrombin time assay at a concentration of 250 µg (Fig. 7). Further, anticoagulant activity of TFS extract was also confirmed by *in vivo* mouse tail bleeding assay. The intravenous injection of TFS extract significantly prolonged the bleeding time in a dose dependent manner and the recorded bleeding time was more than 16 min (960 sec) ($P<0.01$) at a concentration of 10 mg/kg body weight against saline treated control of 2 min 45 sec (165 ± 8 sec) (Fig. 8a). The effect of TFS extract on tail bleeding was completely inhibited by PMSF indicating the role of serine type of protease responsible for the anticoagulant property of the extract (Fig. 8b)

Platelet aggregation

TFS extract inhibited platelet aggregation in a dose Dependent manner. TFS extract at concentrations 50, 100, 150, 200 µg upon incubation with PRP for 15 min inhibited the platelet aggregation by 25, 42, 55 and 65% respectively where ADP used as agonist. The aggregation was monitored

for 6 min (Fig. 9a; 9b). This further confirms the anticoagulant nature of TFS extract.

TFS extract did not showed hemolytic activity upto a concentration of 500 µg while water showed 100% lysis of RBC and saline used as negative control showing no hemolysis. This suggests the non-toxic nature of the extract. Further TFS extract did not showed hemorrhagic and edema inducing activity upto a concentration 200 µg, while *C. gigantia* latex sample induced hemorrhage (18±2 mm) and edema (180±4 of edema ratio) in experimental mice. This suggests the non-toxic nature of the TFS extract.

Discussion

Hemostasis is a regulated physiological response towards damage to blood capillary which stops the blood loss. However, different factors either genetical or environmental may bring imbalance in the hemostatic pathway which may eventually lead to thrombosis. Thrombosis leads to the risk of cardiovascular/cerebrovascular complications which is the major factor of mortality worldwide. Anticoagulant and antiplatelet agents play a major role in treating thrombotic disorders. But side effects of the currently available anticoagulants limit their usage. Several works are being carried out for identification of novel anticoagulant/antiplatelet agents from natural source which are more efficient with less/no side effects. TFS has been reported to exhibit several pharmacological properties, but the anticoagulant property has not been explored [22, 23]. Thus the current study explores the role of TFS extract on thrombotic disorder. TFS extract showed protein bands in non-reduced and reduced condition. Under reduced condition few proteins bands at medium molecular weight and low molecular weight got reduced to lower molecular weight proteins. TFS extract showed bands in PAS staining, suggesting the presence of glycosylated proteins. This is in accordance with the earlier reports of presence of glycosylated proteins in plant system.

TFS extract exhibited proteolytic activity as it hydrolyzed casein at a specific activity of 0.8 U when compared to trypsin with specific activity of 6.1 U. The proteolytic activity was further confirmed by casein and gelatin zymogram assays. The proteolytic activity of TFS extract was completely inhibited by PMSF indicating the presence of serine type of protease in TFS extract.

Serine and cysteine protease have been reported extensively from plant sources [24-26]. While serine and metalloproteases have been reported from ticks, caterpillar, snake venom, spider venom and honey bees [27-29]. TFS extract showed significant anticoagulant activity both in RT and PT assays. In RT the TFS extract increased the clotting time by 2.04 folds while in PT assay it increased the clotting by 1.61 folds. This indicates that TFS extract interfered in extrinsic and common pathway of blood coagulation.

The anticoagulant activity was further evident by *in vivo* tail bleeding assay, in which the TFS extract at 15 mg/kg body weight concentration increased the bleeding time by 6.81 folds. The inhibition of anticoagulant property by PMSF indicates that the showed anticoagulant activity is due to serine protease of the extract. Several anticoagulants proteins have been isolated from different sources such as venoms, insects, plant latex extracts, marine creatures, herbal medicines and sauces [2-29]. Our results are in accordance with the earlier reports and thus may serve as a reliable alternative for use in thrombotic disorders.

TFS extract hydrolyzed all the chains Aα, Bβ and γ of fibrinogen generating low molecular peptides. In general, proteases which hydrolyze fibrinogen from N-terminal end of Aα and Bβ chains generating fibrinopeptides A and B results in procoagulant activity, while the proteases degrade from C-terminal generates truncated molecules results in anticoagulant activity [4, 30, 31]. The *in vitro* anticoagulant activity of TFS extract is supported by *in vivo* tail bleeding study using mouse model.

TFS extract exhibited anti-platelet aggregation property. Platelets are derived from bone marrow megakaryocytes which play a major role in blood coagulant by forming platelet plug at the site of capillary damage. This further forms the blood clot along with fibrin clot. Platelet aggregation was significantly inhibited by TFS extract to an extent of 65% at a concentration of 200 µg. The factors which act as antiplatelet aggregating agents can be a good molecule for inhibiting unusual blood clots. Antiplatelet molecules have been reported from different sources such as snakes, microbes, plants and animals [27-33]. The TFS extract was found to be non-toxic as it did not showed hemolytic activity, hemorrhagic and edema inducing activities.

Tables and Figures

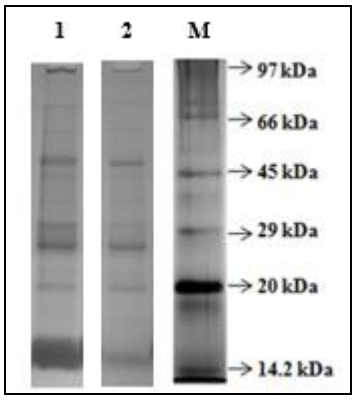


Fig 1: Electrophoresis. TFS extract was loaded onto 10% SDS-PAGE under non-reduced and reduced condition. After electrophoresis the gel was stained with 0.25% coomassie brilliant blue R-250. Lane 1: 100 µg under non-reduced condition, lane 2: 100 µg under reduced condition and lane 3: molecular weight markers.

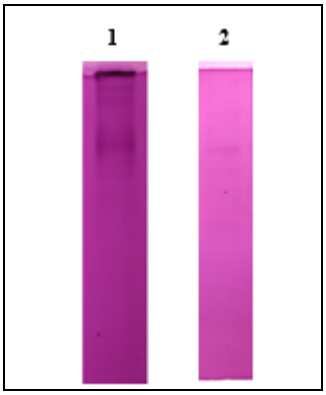


Fig 2: PAS staining. Fibrinogen and TFS extract were loaded onto 10% SDS-PAGE under non-reduced condition. After electrophoresis the gel was stained by PAS staining method for detection of glycoproteins. Lane 1: Fibrinogen (100 µg) lane 2: TFS extract (100 µg).

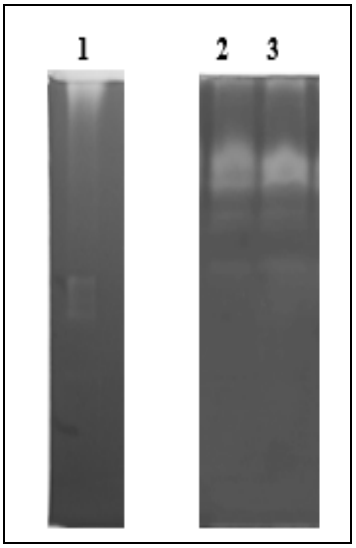


Fig 3: Gelatin zymogram assay. Gelatin (0.2%) was copolymerized with the polyacrylamide gel for the detection of proteolytic activity. The TFS extract was pre-incubated with sample buffer at 37º C for 15 min and loaded onto 10% SDS-PAGE under non-reducing condition. After electrophoresis gels were washed with 2.5% of Triton X-100 for 1h to remove SDS. The gels were incubated overnight in incubation with buffer. Lane 1: Trypsin (5 µg), lane 2 and 3: TF extract 10 and 20 µg.

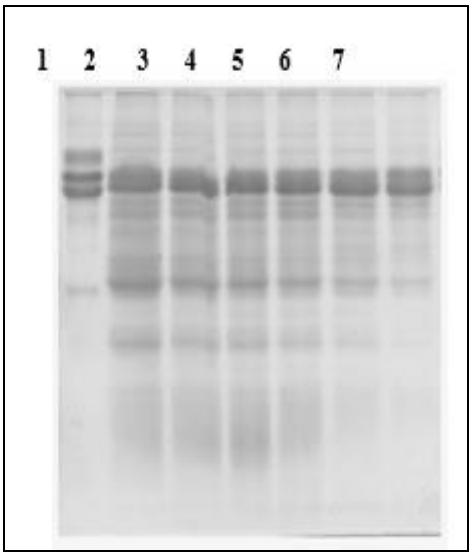


Fig 4: Dose dependent fibrinogenolytic activity. Human fibrinogen (50 µg) was incubated with different concentrations of TFS extract (0–30 µg) at 37℃ for 4 h. The reaction mixtures were subjected to SDS-PAGE (12%) under reducing conditions in order to visualize the degradation patterns. Lane 1: fibrinogen; lanes 2–7: fibrinogen incubated with 5, 10, 15, 20, 25 and 30 µg of TFS extract, respectively.

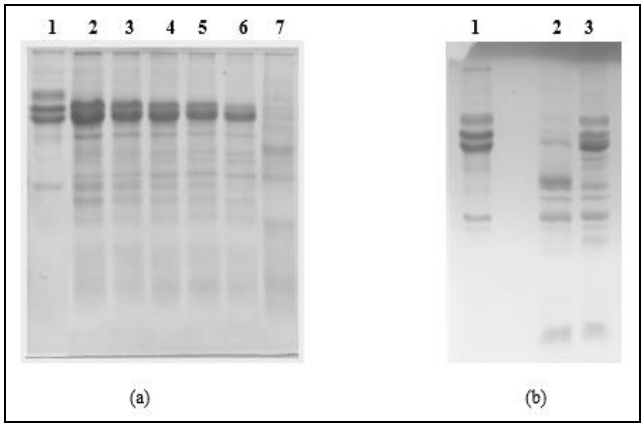


Fig 5: (a) Time dependent fibrinogenolytic activity. Human fibrinogen (50 µg) was incubated with TFS extract (50 µg) for different time at 37℃. The reaction mixtures were subjected to SDS-PAGE (12%) under reducing conditions in order to visualize the degradation patterns. Lane 1: fibrinogen; lanes 2–7: incubated for 2, 4, 8, 12, 16 and 24 h with TFS extract, respectively. (b) Effect of inhibitor on fibrinogenolytic activity. Lane 1: Human fibrinogen alone, lane 2: fibrinogen incubated with TFS extract for 24 h, lane 3: fibrinogen incubated with TFS which was preincubated with PMSF (5 mM) for 15 min at 37º C.

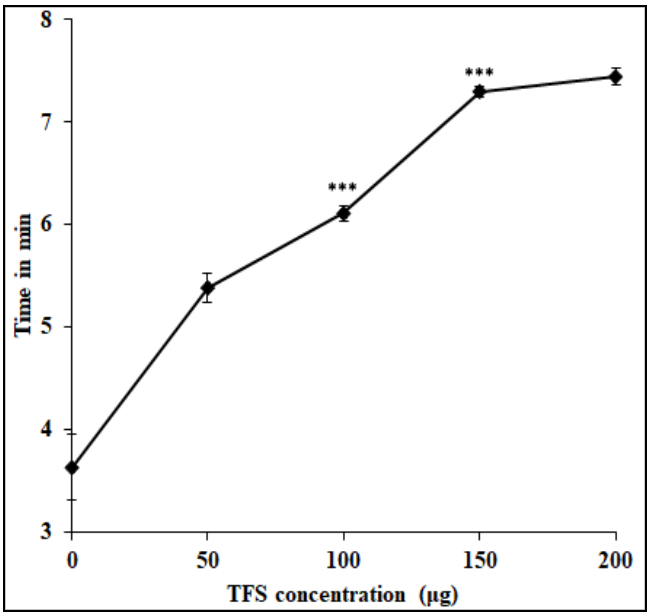


Fig 6: Recalcification time (RT). (a) Different concentration of TFS extract (50 - 200 µg/20 µl) was incubated with human citrated plasma (100 µl) for 5 min. After incubation 25 mM $CaCl_2$ (100 µl) was pipette and clotting time was recorded. Values are presented as mean ± standard error of the mean (n=3). Statistically significant results are indicated by asterisks, *** – $p < 0.001$.

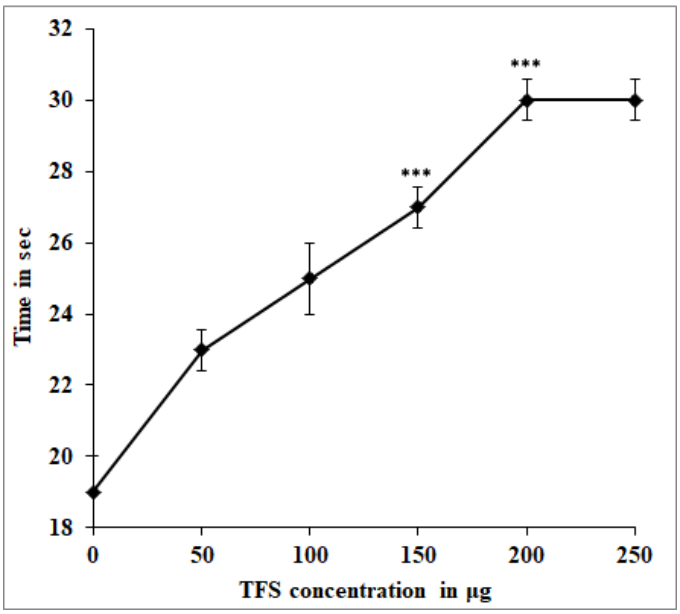
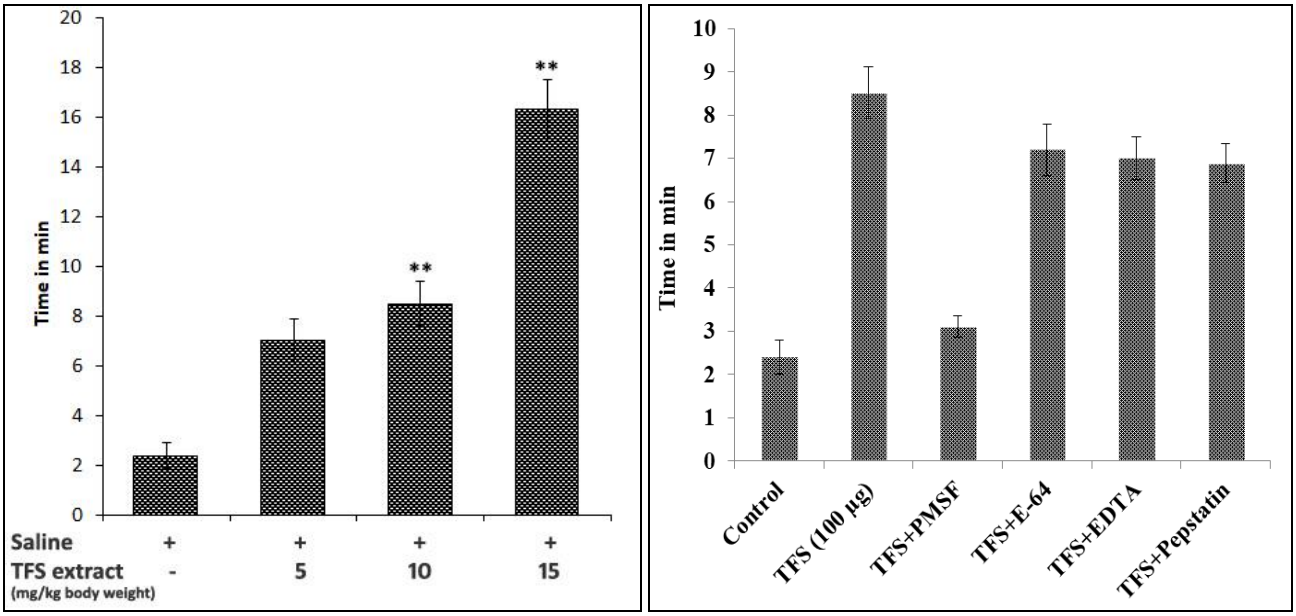


Fig 7: Prothrombin time (PT). Different concentration of TFS extract (50 - 250 µg/20 µl) was incubated with human citrated plasma (100 µl) for 5 min. After incubation 200 µl of uniplastin was added and clotting time was recorded. Values are presented as mean ± standard error of the mean (n=3). Statistically significant results are indicated by asterisks, *** – $p < 0.001$.



(a) (b)

Fig 8: Effect of TFS extract on bleeding time. (a) Tail bleeding was measured 10 min after the intravenous injection of TFS extract. (b) TFS was preincubated with 5 mM of PMSF, E-64, EDTA and pepstatin for 15 min at 37 ºC. Each value represents mean ± SD of three independent experiments (** - p<0.01). TFS extract, *Trigonella foenum-graecum* sprouted seed aqueous extract.

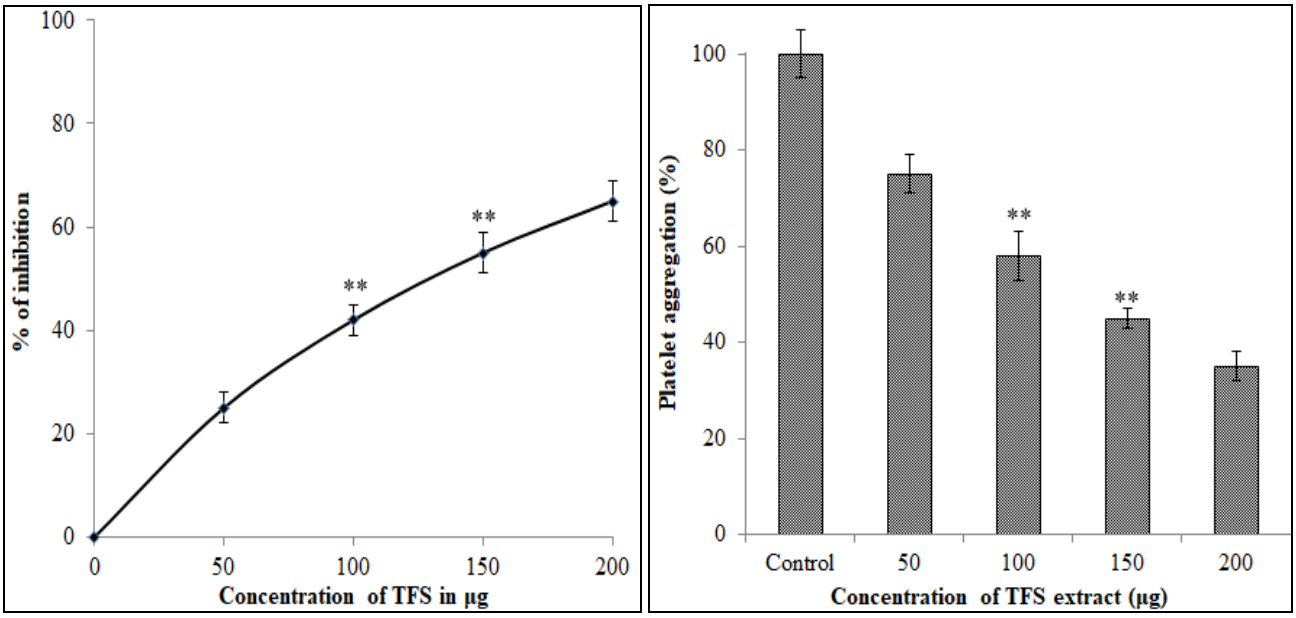


Fig 9: Effect of TFS extract on platelet aggregation. Platelet aggregation was monitored in a Chronolog Model 700 aggregometer. Platelet-rich plasma was incubated with TFS extract for 15 min and aggregation was initiated by adding ADP. The aggregation was monitored for 8 min. All the values are presented as mean ± standard error of the mean (n=5). (a) The percentage of inhibition of platelet aggregation with TFS extract; (b) the percentage of platelet aggregation in the presence of TFS extract. Statistically significant results are indicated by asteriks ** – $p<0.01$.

Table 1: Protease inhibition

Inhibitor	% of inhibition
Control	0
E-64	8
PMSF	90
EDTA	0
Pepstatin A	0

Conclusion

In conclusion, the current study reports for the first time the anticoagulant and fibrinogenolytic activities in TFS extract. The exhibited anticoagulant activity is due to the proteolytic activity with serine proteases. Further isolation and evaluation of the active molecule form TFS extract may give a molecular insight into its mode of action and can further be used to treat thrombotic disorders.

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