



Thrombolytic and allelopathic activities of medicinal plant: *Strobilanthes urticifolia* Wall. Ex Kuntze.

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Key words: *Strobilanthes urticifolia*, Thrombolytic, Allelopathic, CME, SK, Fractions

<http://dx.doi.org/10.12692/ijb/11.6.39-44>

Article published on December 12, 2017

Abstract

Indigenous knowledge and literature review reveals that there is no such information or citation is available on proposed study and was aimed to evaluate the crude methanolic extract (CME) and different fractions of *Strobilanthes urticifolia* Wall. ex Kuntze for thrombolytic and allelopathic activities. CME and different fractions were tested against clot lytic activity in the thrombolytic test and the result showed that crude methanolic extract (56.11%) and chloroform fraction (64.94%) have highest thrombolytic activity followed by n-hexane fraction (29.66%) while effect of ethyl acetate fraction (7.58%) and aqueous fraction (9.88%) on the blood clots were almost negligible as compared to positive control streptokinase (SK) and negative control water which showed 76.44% and 5.97 % lysis of clot respectively. Only aqueous fraction was checked for the presence of allelochemicals in allelopathic activity and the result showed that at low concentration (25 and 50 %) the germination was 70±3.50% and 60±2.00% respectively while at high concentration of aqueous fraction it was reduced to 40±3.50 as compared to negative control water. Moisture content, shoot and radical length of the wheat seed in allelopathic activity was also noted and it also reduced in dose-dependent (25, 50 and 100 %) manner. These results suggest that CME and different fractions of *Strobilanthes urticifolia* Wall. ex Kuntze has significant thrombolytic and allelopathic properties and needs further exploration.

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Introduction

Thrombus is a dangerous form of blood clot because it forms in a healthy blood vessel and impedes the function of a blood vessel therefore despoil tissues of regular blood movement and oxygen (Ali *et al.*, 2014). This consequence cause necrosis of the tissue in that area (Laurence and Bennett, 1992). Thrombolysis, also called thrombolytic therapy, is a treatment to dissolve risky clots in blood vessels, improve blood flow and prevent damage to tissues and organs (Zaman *et al.*, 2015). Dealing with the coronary arteries blood clotting, streptokinase (SK) and urokinase (UK) or tissue plasminogen activators are widely applied as first choice clinical thrombolytic agents (Ansaria *et al.*, 2016). In addition, the above agents also solve the problems of severe deep vein thrombosis, pulmonary embolism, myocardial infarction, and occluded intravenous. However, practice of these medications remains highly observed to alter the related complications, including intracranial blood bleeding, hypersensitive reaction, and loss of specificity (Rahman *et al.*, 2013). Also, these medicines are not applied on patients who had experienced surgery or individuals with a history of nervous lesions, gastrointestinal bleeding, or hypertension (Naderi *et al.*, 2005). Hence, investigating for novel therapeutic agents may allow obtaining new medicines with better pharmacological effects, extended range of remedies, effectiveness, and immunity.

Plants are important source of secondary metabolites/chemicals which have good or harming effect on the growth of other plants, this phenomenon is termed as allelopathy (Hien *et al.*, 2015) and the chemicals are called allelochemicals which are present in all parts of plants (Putnam and Tang, 1986). Allelochemicals of plants are used in the control of weeds; an undesired plant, which impinge the development and production of agriculture crops (Asaduzzaman *et al.*, 2014). The Plants having such allelochemicals are natural substitute of herbicides which will show good effect on environment (Cantrell *et al.*, 2012; Khang *et al.*, 2016).

There is little or no such reference is available about medicinal values of *Strobilanthes urticifolia* Wall. ex Kuntze and no studies have yet been reported on its thrombolytic and allelopathic activities. Therefore, the objective of this research was to examine the thrombolytic and allelopathic activities of the crude methanolic extract and different fractions from *Strobilanthes urticifolia* Wall. ex Kuntze.

Materials and methods

Plant materials

The plant was identified in the Department of Botany, University of Peshawar, Pakistan. The whole plant was collected in 2016 from the Northern region of Pakistan.

Preparation of plant extract

The plant material was shade dried, chopped and grinded into fine powdered. Then extraction of powdered plant (12 kg) was done with methanol for 15 days at room temperature, twice, with occasional shaking. After filtration, filtrate was concentrated in a rotary evaporator which was set at 40 °C to produce methanolic extract.

Fractionation

The crude methanolic extract (880 g) was liquefied in distilled water (500 ml) and partitioned with different polar and non-polar solvents such as n-hexane, chloroform and ethyl acetate to produce n-hexane (250 g), CHCl₃(220 g), EtOAc (85 g) and aqueous (325 g) fractions.

In vitro thrombolytic activity

Thrombolytic activity was performed according to the procedure defined earlier (Ansaria *et al.*, 2016; Prasad *et al.*, 2007; Kabir *et al.*, 2015; Prasad *et al.*, 2006). About 6ml blood drawn from the healthy volunteer through sterile syringe without a history of oral contraceptive or anticoagulant therapy, was distributed in six pre-weighted sterile microcentrifuge tubes (0.5 mL/tube) and incubated at 37 °C for 45 min. After clot formation, the serum was completely discarded without disturbing the clot, and each tube having clot was again weighed to determine the clot

weight (clot weight=weight of clot-containing tube–weight of tube alone). 100µl from each sample (10 mg/mL) was added to each microcentrifuge tube separately containing pre-weighed clot. Streptokinase (100µl) and distilled water (100µl) were used as positive and negative control respectively and separately added to the control tubes. All the tubes were then incubated at 37 °C for 90 min and observed the RBCs for clot lysis. After incubation, the tubes were inverted to remove any fluid formed and the tubes were again weighed to see the change in weight after clot disruption. The variance in weight before and later clot lysis was expressed as the percentage of clot lysis, as shown below:

$$\% \text{Degradation} = \frac{\text{Weight of clot degrade}}{\text{Net weight of clot}} \times 100$$

Allelopathic activity

Allelopathic activity was checked against wheat seeds for the existence of allelochemicals by using aqueous fraction (Rawat *et al.*, 2016). Stock solution of the aqueous fraction was got ready by mixing 20g in 100ml distilled water. From the stock solution, doses of different amount (25%, 50% and 100%) were used on the wheat seeds to check their effect on germination, shoot and radical length, fresh and dry weight and moisture contents.

Results and discussion

Thrombolytic activity

Crude methanolic extract along with various fractions of *S. urticifolia* were evaluate for thrombolytic activity. Results are summarized in Fig. 1.

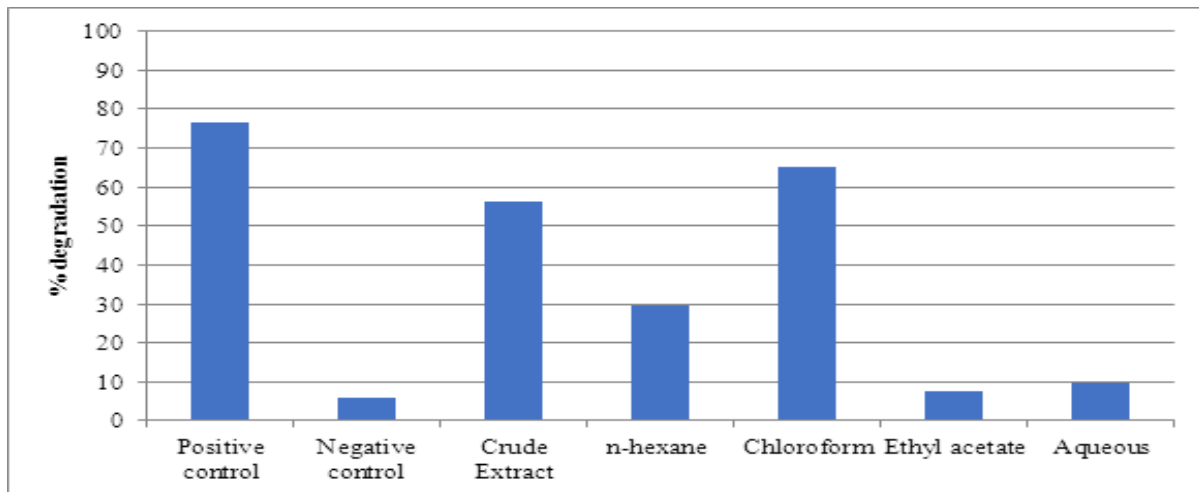


Fig. 1. Antithrombotic effect of test sample.

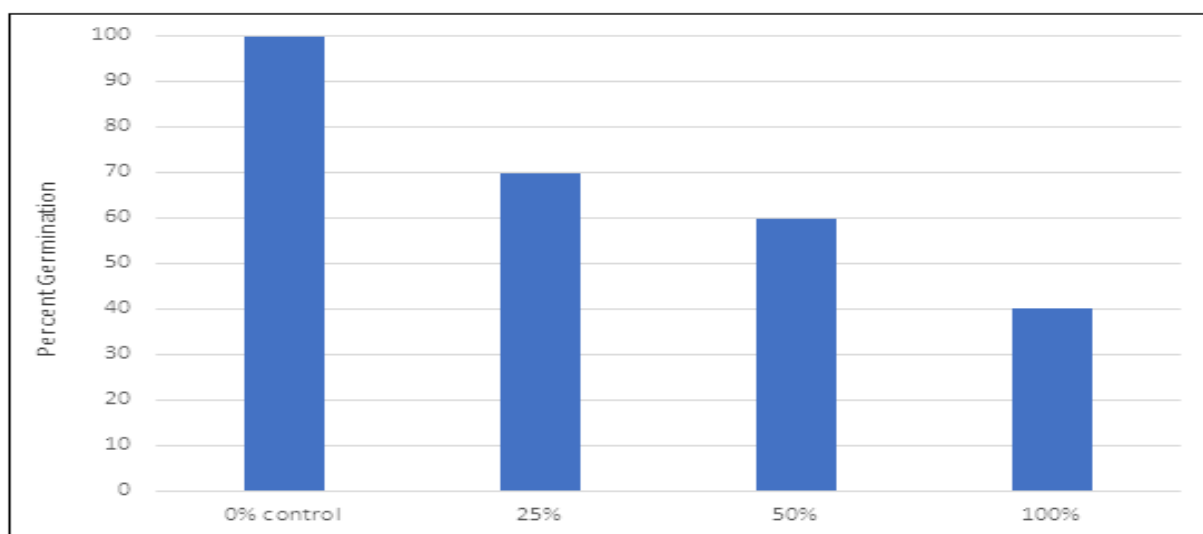


Fig. 2. Percentage germination of seeds.

Streptokinase (100 μ l) was used as a positive control and lysed 76.44% clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (5.97%). The CME and chloroform fraction showed good activity i.e. 64.94% and 48.15% respectively.

The *n*-hexane fraction, showed low activity (29.66%). While effect of ethyl acetate and aqueous fractions were almost negligible i.e. 7.58% and 9.88% respectively. The result showed that the plant contains antithrombosis agent and needs further exploration for thrombolytic therapy.

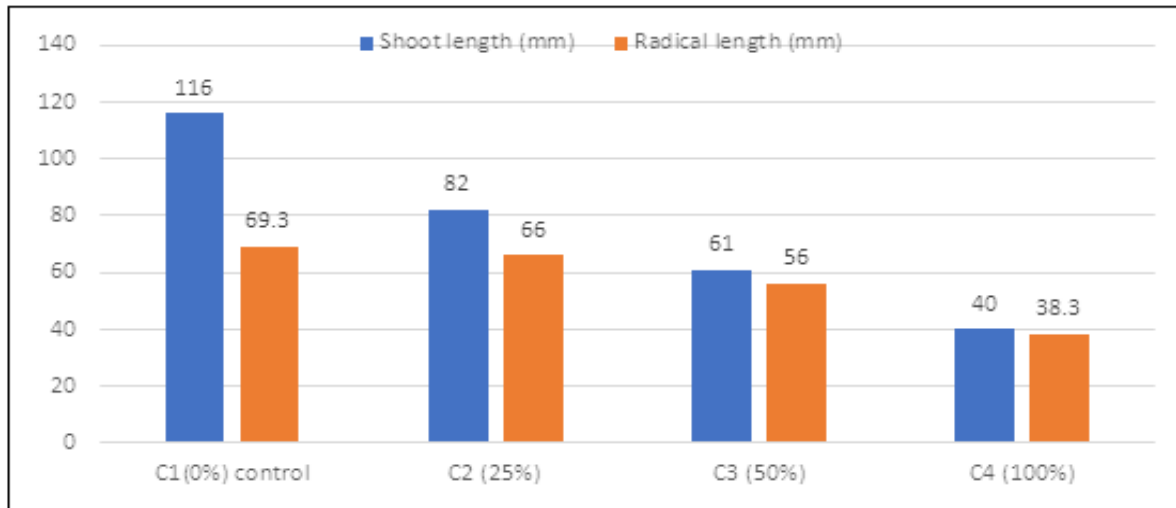


Fig. 3. Growth inhibitions of shoot and radical length.

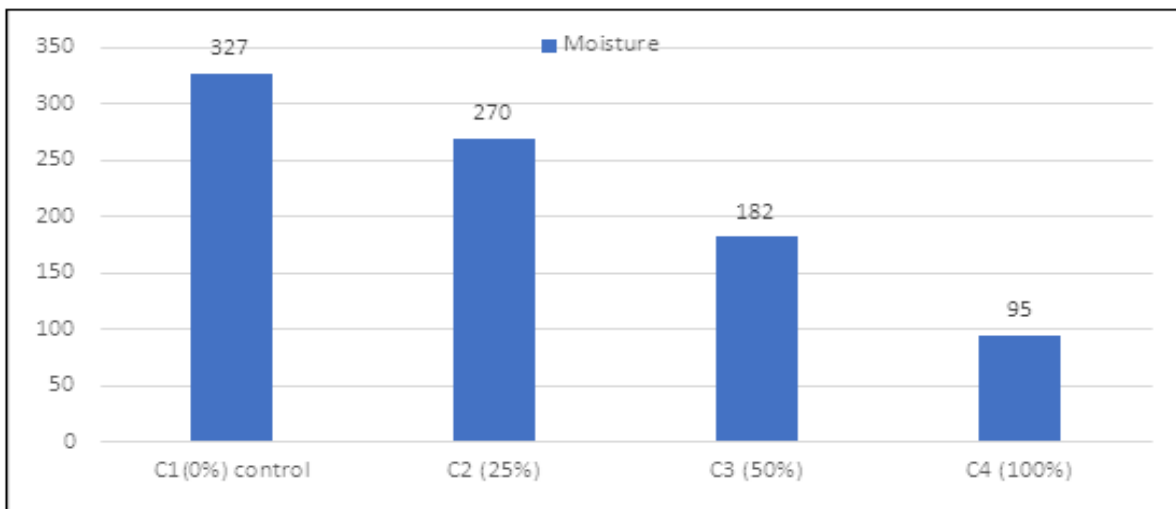


Fig. 4. Moisture content.

Allelopathic activity

The results showed that the germination of the wheat seed inhibited as the concentration of the test sample increased as presented in Figure 2. At 100% concentration, germination was $40 \pm 3.50\%$ while it was increased to $60 \pm 2.00\%$ and $70 \pm 3.50\%$ after the concentration was reduced to 50% and 25% respectively as compared to negative control (water) where the seed grown 100%.

The shoot and radical length of wheat seed was also being reduced in dose dependent manner. At 100% dose, the length of shoot and radical was 40.5 ± 2.50 mm and 38.3 ± 4.0 mm while it was increased when the amount was reduced to 50% and 25% respectively as compared to water (negative control) as showed in Fig. 3. This activity represents that the test sample has not ably repressed the germination as well as shoot and root length of the wheat seed.

Moisture content of the seeds at different concentrations was also worked out which is the variance of fresh weight and dehydrated weight. In the negative control (water) the moisture content was 327+ 3.13mg while it was decrease to 270+ 4.0mg and 182+ 4.00mg when the amount of the sample was increased to 25% and 50% respectively. At 100% amount, the moisture content reduced to 95+ 3.50 mg as showed in Fig. 4. The result showed that the moisture content of the seeds reduced when the concentration of the test samples increased.

Conclusion

From the above results, it can be concluded that *S. urticifolia* has the potential to be used as an antithrombotic agent after proper processing. In addition, the aqueous fraction possessed significant allelopathic activity and contained allelochemicals which affect different hormones of the wheat seeds like auxins and cytokinins thus dropping its growth. Further studies are necessary to confirm traditional use of this plant.

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