Evaluation on In Vitro Blood Clot Dissolving Potential of Aqueous Extract of Sida acuta Burm. F. Leaves

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Abstract

Clotting of blood is the vital processes and a perplexing interaction of various mechanisms of circulatory system due of failure of which is sometimes considered as a concern within the circulatory system causing acute myocardial or cerebral infarction which might cause demise. *Sida acuta* Burm. f. (Malvaceae) is abundantly growing small perennial shrub utilized by nates for diuretic, anthelmintic, calmative and wound healing properties, and are utilized in treating disorders like blood, bile, liver, nervous, urinary diseases and rheumatism. The present study was intended to evaluate the blood clot dissolving potential of *Sida acuta* leaf aqueous extract in *vita*. The plant material as leaves were locally collected and subjected to phytochemical extraction with chilled water. The preliminary phytochemical test total phenolic content was estimated by Folin-Ciocalteu’s method. *In vitro* thrombolytic activity of 3 different concentrations of aqueous extract was estimated on goat blood clot compared to the activity of streptokinase. The aqueous extract of *S. acuta* leaves are reported to be rich in alkaloids, flavonoids, tannins, terpenoids and glycosides while the total phenolic content was estimated to be 17.48% in extract which are mostly responsible for any pharmacological activity. Compared to the thrombolytic activity of standard streptokinase which was 73%, the aqueous leaf extract of *S. acuta* displayed considerable blood clot dissolving activity at concentration 10 mg/100µl, 5.0 mg/100µl, and 2.5 mg/100µl as 41%, 34% and 12% respectively. This property of plant extract is promising which could be could be exploited in development of new biopharmaceutical and therapeutic agents after stringent further physiological compatibility and in *vivo* pharmacological studies.

Keywords: *Sida acuta*, phytochemical extract, thrombolytic activity, streptokinase

INTRODUCTION

Blood clotting is one of the vital processes that takes place in humans, animals and birds. Clotting of blood is a perplexing interaction of various mechanisms, where enactment of the coagulation, fibrinolytic frameworks, disruption of the vascular endothelium, and the generalized triggering of cellular mechanisms prompts to clotting on the surface of monocytes and platelets available in flow. Sometimes blood clots are considered as a concern when formed in the circulatory system due to disturbances in hemostasis resulting in vascular obstruction and causating consequences in thrombotic diseases like acute myocardial or cerebral infarction which might cause demise. Hereditary factors, primary or acquired, play a role in the development of thrombosis. In pharmacological terms the dissolution of blood clot is regarded as thrombolytically achieved by secondary fibrinolysis by plasmin through application of tissue plasminogen activator equivalents which are plasmin activator proteins. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator are available in the market as clot dissolving agents. Due to certain negative and life-threatening side effects anticoagulants currently in use scientists and are interested in finding new alternatives to these anticoagulants of natural and organic origin.

Medicinal plants are the premier source of drugs and pharmacological components as phytotherapy, derived from different parts of the plants. *Sida acuta* Burm. f. (Malvaceae) is one of those plants as of now utilized by native individual to manage health issues. This plant is an erect, small perennial herb or small shrub of about 1.5 m height with branches. It grows abundantly on squander regions, cultivated fields and roadsides.

As a therapeutic all parts of the *S. acuta* are used, however the leaves are the most often demand. Leaves are considered to havediuretic, anthelmintic, calmative and wound healing properties, and are utilized in treating rheumatism. Stomach torment, hemorrhoids, azoospermia and oligospermia are being used to treat with leaf decoctions of this plant. While, vomiting and gastric disorders were controlled using its leaf juice. Roots are mostly used as a stomachic, diaphoretic and antipyretic in Indian traditional...
medicine. *S. acuta* also used for disorders like blood, bile, liver, nervous and urinary diseases, while the hot water extract of whole dried plant is orally used as febrifuge, and abortifacient.12, 13 *Sida acuta* is a constituent in Siddha formulation recommended in pulmonary tuberculosis, rheumatism, facial paralysis, sciatica, haemorrhage, spermatorrhoea, leukorrhoea and gonorrhoea.14 Folks say this plant also have thrombolytic activity to some extent. So, the present work was aimed at phytochemical investigation and blood clot dissolving activity of aqueous extract of leaves of *Sida acuta* under *in vitro* conditions.

**MATERIALS AND METHODS**

**Sample Collection and Processing**

The plant material as leaves of *Sida acuta* (Burm.) were collected from roadside in Bhopal City and were authenticated by botanists and used as raw material. The collected leaves were thoroughly washed in tap water, drained and allowed to dry at room temperature. The dried leaves were then grounded into fine powder followed by defatting with petroleum ether overnight at ambient temperature.

**Phytochemical Extraction and Analysis**

The defatted leaf powder of *S. acuta* plant was then subjected to phytochemical extraction with pure distilled water by soxhlet method in order to prepare their aqueous extract. The obtained aqueous extract was then concentrated by evaporating the solvent in a boiling water bath. The preliminary phytochemical analysis was done according to the methods described by Harborne,15 Khandelwal16, and Tenguria et al.,8 which are described as follows:

a. **Test for alkaloids**: Few drops of Dragendorff's reagent was mixed in diluted stock of extract if yields orange red precipitate demonstrates the presence of alkaloids.

b. **Test for tannins**: Small amount of diluted extract stockis when warmed and followed by addition of 2-3 drops ferric chloride solution gives a dark green colour in solution demonstrates the existence of tannins in extract.

c. **Test for terpenoids**: About 100 μl of stock was diluted with distilled water in a test tube and carefully 2 ml chloroform (CHCl₃) was added to it by the side wall of test tube followed by addition of concentrated H₂SO₄ (3ml) in the same way to form a layer. The formation of a reddish brown coloration or a ring at the interface is the indicator for the presence of terpenoids in extract.

d. **Test for saponins**: The diluted stock of extract when warmed a little then had shaked vigorously. The formation of froth or bubbles that stays for 5 minutes at least indicates the presence of saponins.

e. **Test for flavonoids**: To the diluted stock of extract in a test tube 2-3 drops of 10% lead acetate was added. The appearance of a creamy white dirty precipitate demonstrates the presence of flavonoids in extract.

f. **Test for glycosides**: Benedict reagent was used to check the presence of glycosides in diluted extract. The diluted extract was first warmed they 2-3 drops of Benedict's reagent were added to the reaction tube. The appearance of yellow or orange precipitate indicates the presence of glycoside in extract.

**Total Phenolic Content**: The total phenolic content (TPC) of the extract was assessed through Folin-Ciocalteu method with suitable modification.17, 18 The suitably diluted extract was made up to 3 ml with distilled water and was oxidized 0.5 ml of with Folin-Ciocalteu reagent, and the reaction was neutralized by addition of 2 ml of 20% sodium carbonate solution. The reaction was permitted to stand for a 60 min in the dark at room temperature, and absorbance of the resulting blue colour was measured at 560 nm. The TPC was evolved from the calibration curve of gallic acid (using concentrations 0.2 mg/ml), and the outcomes were stated as mg of gallic acid equivalent per g dry weight was expressed as mg GA equivalent/L of extract.

**In vitro Clot Dissolving Activity**

*In vitro* experiment was thrombolytic activity was planned for present investigation according to the methods suggested by Sweta et al., (2007), Fatema et al., (2017) and Alawa, et al., (2018) with suitable modification as per present study.19, 4, 5 The goat blood samples were collected from local slaughter house and 0.5 ml of poured into 5 different 1.5 ml microfuge and incubated at 37°C for 3 hours to allow the clot formation followed by removal of serum carefully and weight of the clot was measured. Three serial dilutions were made from the 100 mg/ml stock solution of aqueous extract of *S. acuta* was. With the help of micropipette, 100 μl of aqueous extract from each dilution was added in each corresponding separated microfuge touching the surface of blood clot. Streptokinase and sterile distilled water were used in each separate microfuge with blood clot was used as positive and negative control respectively. The set of experiment was incubated at 37°C for 2 hours followed by observation *in vitro* thrombolytic activity. The liquid released after incubation was drained and residual clot was washed with sterile distilled water and moisture was removed by drying. Final weights of microfuge were taken to determine the percentage thrombolytic activity of extract compared to standard through following formulae;

\[
\text{% Thrombolysis} = \frac{\text{Weight of clot after incubation} - \text{Weight of clot before incubation}}{\text{Weight of clot before incubation}} \times 100
\]

**RESULTS AND DISCUSSION**

**Preliminary Phytochemical Analysis**

The results of phytochemical analysis of aqueous extract of *S. acuta* leaves are depicted in table 1 which indicates the aqueous extract is rich in phyt constituents like alkaloids, flavonoids, tannins, terpenoids and glycosides. Though saponins were reported to be absent in aqueous extracts.

**Table 1: Phytochemical Analysis of *Sida acuta* (Burm) leaf aqueous extracts**

| S.N. | Constituents Tested | Aqueous Extract of *S. acuta* (Burm) leaves |
|------|---------------------|--------------------------------------------|
| 1    | Alkaloids           | +4                                         |
| 2    | Flavonoids          | +4                                         |
| 3    | Tannins             | +3                                         |
| 4    | Saponins            | -                                          |
| 5    | Terpenoids          | +2                                         |
| 6    | Glycosides          | +4                                         |

(+) means present, (&) means absent

In an earlier investigation Senthil kumar et al., (2018) also reported more or less similar phytochemical profile for aqueous extract of *Sida acuta* leaves.20 Various phytoconstituents chiefly polyphenols and flavonoids are
responsible for the biological and pharmacological activity of medicinal plants.6,9

Estimation Total Phenolic Content

In present investigation 20 times diluted 100 mg/ml stock solution of aqueous extract of S. acuta leaves gave an absorbance reading of 0.830 in reaction mixture in digital micro processed spectrophotometer (Electronic India model EI-2305) when subjected to estimation of phenolic content through calibration curve of standard plot with phenol equivalent to gallic acid (table 2 and figure 1) indicates the presence of 17.48 mg/100 mg aqueous extract of S. acuta leaves or simply the total phenolic content in aqueous extract of S. acuta leaves was reported to be 17.48 %. Most of the earlier investigation reported TPC in extracts other than aqueous extracts. Muneeswari et al. (2019) reported 31±0.15 mg/g TPC in ethanolic extract of S. acuta leaves. The presence of tannins and phenolics are responsible of the anti-inflammatory, antimicrobial and free radical scavenging property of any medicinal plant or their extracts.22,23,24

Table 2: Gallic acid as standard concentration vs absorbance at 650 nm to plot standard curve for estimation of phenolics in samples Using Folin-Coeucalut’s Method.

| S.N. | GA Concentration in mg/ml | Absorbance at 650 nm |
|------|---------------------------|---------------------|
| 1    | 2                         | 1.891               |
| 2    | 1                         | 0.976               |
| 3    | 0.5                       | 0.457               |
| 4    | 0.25                      | 0.228               |
| 5    | 0.125                     | 0.128               |

In present investigation the in vitro thrombolytic activity of aqueous leaf extract of S. acuta at a concentration of 10 mg/100µl solution is reported to be 41 % which reduces to 34 % and 12 % when 5.0 mg/100µl and 2.5 5.0 mg/100µl extract solution were used respectively. Streptokinase at a concentration of 0.1 mg/ml generally produces a thrombolytic activity in range from 80 to 95 %.25,26 In present study, Streptokinase displayed an activity of 73% as positive control at similar concentration that is optimum. The activity of varied concentration of aqueous extract of S. acuta leaves and streptokinase, where the dissolution of blood clot due to extracts is considerable (Figure 2).

In terms of blood related studies, Eze, and Nwodo, (2016) reported the significant inhibition of in vitro haemolysis while investigating the potentiality of membrane stability; platelet aggregation and activities of phospholipase A and prostaglandin in ethanol extract leaves of Sida acuta.27 While working on methanolic extract S. acuta leaves, Bahar, et al., (2013) reported 24.786 % of thrombolytic activity using 100 µl of 1 mg/ml and 0.5 mg/ml concentration.28 Heavy external blood clots due to any tissue tear or rupture or large accidental wound development is also a problem sometimes when causing painful bandage. Though in present study the thrombolytic activity is reported at higher concentrations, but it is easy and cost effective to obtain the aqueous extracts of any plant material.

Table 3: In vitro clot dissolving activity of aqueous extract of S. acuta leaves compared to gel containing Streptokinase

| S. No. | Test Samples | Designation       | Concentration | Percentage Clot Dissolving Activity |
|--------|--------------|-------------------|---------------|-----------------------------------|
| 1      | C-1          | -ve Control with water | NA            | 1.4%                              |
| 2      | C-2          | +ve Control with Streptokinase | 0.01 mg       | 73%                               |
| 3      | D-1          | Dilution 1 of extract | 10.0 mg       | 41%                               |
| 4      | D-2          | Dilution 2 of extract | 5.0 mg        | 34%                               |
| 5      | D-3          | Dilution 3 of extract | 2.5 mg        | 12%                               |

Figure 1: Standard Plot for known concentration of Gallic acid Standard at 650 nm. The Graph is obtained from Excel 2013 linear regression function

In vitro Clot Dissolving Activity

The in vitro clot dissolving activity of aqueous extract of test plant considered in present study indicates the encouraging outcomes in prospect of developing new therapeutic substances. There were 3 different concentrations of extracts used whose percentage blood clot dissolving activity are illustrated in table 3 compared to the standard streptokinase.

Figure 2: Graphical representation of in vitro blood clot dissolving potential of S. acuta aqueous leaf extract and standard streptokinase.
CONCLUSION

The outcomes of present investigation indicates that the aqueous extract of S. acuta leaves is significantly rich in phenolic contents and variety of phytochemical constituents that could be explored for pharmacological properties in order to develop new drug for the cure of variety of pathological conditions in humans and animals. In present investigation the aqueous extract of S. acuta leaves were observed to be promising sources of thrombolytic drug under in vitro experiments. After this preliminary study, further extensive and stringent studies related to their cytotoxic & genotoxic studies, and physiological compatibility and in vivo pharmacological studies, the thrombolytic property of aqueous extract of S. acuta leaves could be exploited in development of new biopharmaceutical and therapeutic agents which could be easily accessible, cost effective and probably safe due to their natural origin.

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