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Research Article

EVALUATION OF *IN VITRO* THROMBOLYTIC AND ANTIPROTEINASE ACTIVITIES OF *WEDELIA TRILOBATA* (LINN.)

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ABSTRACT

Objective: The objective of the study was to evaluate the phytoconstituents and *in vitro* thrombolytic and antiproteinase activities of aqueous extracts of leaf, stem, and root of *Wedelia trilobata*.

Methods: *In vitro* thrombolytic activity of aqueous extracts of different parts of *W. trilobata* against thrombus. *In vitro* antiproteinase activity of aqueous extracts of different parts of *W. trilobata.*

Results: Phytochemical screening of aqueous extracts of leaf, stem, and flower of *W. trilobata* showed various bioactive constituents such as carbohydrates, protein, phenols, tannins, saponins, alkaloids, terpenoids, and quinines in all three parts of aqueous extracts. The maximum fibrinolysis (blood clot lysis) was observed in leaf extracts when compared to stem and extracts of flower *W. trilobata*. The highest degree of proteinase activity was showed in stem of *W. trilobata.*

Conclusions: Hence, the aqueous extracts of a different part of *W. trilobata* possess many phytocompounds may be responsible for thrombolytic and antiproteinase activities. In further, to isolate the particular phytocompounds from this plant may be substantiate an effective drug in pharmacologic aspect as thrombolytic and anti-inflammatory therapy.

Keywords: *Wedelia trilobata*, Leaf, Stem, Flower, Aqueous extracts.

INTRODUCTION

Herbal plants have always been considered as a healthy resource of life for all people. Therapeutically properties of medical plants are very useful remedies in various diseases, and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombard with thousands of unhealthy products, the level of sensibility in front of diseases is very high and the use of medicinal plants can characterize the best solution [1]. A medicinal plant is one or more bioactive constituents that can be used for beneficial resolution and also used as a precursor for the production of drugs [2]. Herbal products are extensively perceived as safe because they are "natural" [3] having less or no side effects.

Thrombosis is the important pathophysiological process that underlies the acute coronary disorders such as pulmonary emboli, deep vein thrombosis, strokes, and heart attacks; which are the main causes of morbidity and mortality in developed countries [4]. This disease is characterized by the development of a blood clot (thrombus) in the circulatory system of the body due to the failure of homeostasis which leads to vascular blockage and while recovering causes fatal significances, myocardial or cerebral infarction, as well as death [5]. Therefore, anticoagulation treatment is the basis of management, and the proper choice of thrombolytic drugs to decrease platelet aggregation. Intravenous heparin, the first line of the treatment for cerebral venous sinus thrombosis, is used in the anticoagulation therapy because it is safe, effective and feasible. The thrombolytic agents are anistreplase, streptokinase, alteplase, tissue plasminogen activator, and urokinase [3].

Inflammation is a local response of living mammalian tissues to injury. It is a body defense reaction to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury and pain. Even though most of the synthetic anti-inflammatory drugs are accessible in the market, due to their well-known side effects, harmfulness and manufacture cost are higher. In many inflammatory disorders, there is excessive activation of phagocytes, production of O_2^-,OH^- radicals as well as non free radicals species (H_2O_2) [6]. Drugs from plant origin are relied on by 80% of the world's population. In India, the use of herbal drugs is an important component of the traditional system of medicine.

Wedelia trilobata is a flowering plant of sunflower family, Asteraceae. A tropical perennial medicinal herb, with deeply lobed fleshy leaves and flowers are orange - yellow. *W. trilobata* is a medicinal plant used to treat hepatitis infections, to clear the placenta after birth and is used for menstrual pain and unspecified female complaints [7]. *W. trilobata* is various biological activities, such as antidiabetic, antibacterial, antitumor, hepatoprotective, and central nervous system depressant properties [8]. Hence, the present study aimed to evaluate the phytochemical, *in vitro* thrombolytic and anti-inflammatory potential of aqueous extracts of three different parts (leaf, stem, and flower) of *W. trilobata* (L.).

METHODS

Collection of plant materials

Plant of *W. trilobata* was collected from Mayiladuthurai, Nagapattinam (District), Tamil Nadu, India, during November 2016. The plant was identified and authenticated by Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The leaf, stem, and flowers of plant materials were separated and washed thoroughly in tap water followed by distilled water. The parts of plant were shade dried under the room temperature for 7 days after plant materials were uniformly grinded well using a mechanical grinder. The powder materials were stored in air tight container.

Preparation of extracts

To one part of powder plant materials and three parts of distilled water was added separately and boiling the contents thoroughly to reduce the

original volume. After boiling the contents were filter through muslin clothes and then the filtrate was evaporated to boiling water bath. A paste form of extracts was store separated in airtight containers at 4°C, which was subjected to various analyses.

Qualitative method of phytochemical analysis

The leaf, stem, and flower of *W. trilobata* extract alkaloids, carbohydrates, glycosides, protein, steroids, tannins, phenols, flavonoids coumarins, saponins, quinone, terpenoids, phlobatannins, and anthraquinones were analyzed by standard procedure [2].

***In vitro* thrombolytic activity**

In vitro thrombolytic activity was carried out by Prasad *et al*. [9]. Venous blood samples (3 ml) were drawn from healthy human volunteer. 200 µl of blood was transferred to each of previously weighed Eppendorf tubes for each subject. In the first series, the transferred 200 µl allowed to form clots at 37°C for 45 minutes. After clot formation, serum was completely removed and each tube having clot was again weighed to determine the clot weight (clot weight=weight of clot containing tube−weight of the tube alone). To each Eppendorf containing pre-weighed clot, 200-1000 µl of different concentration of plant extracts, or 100 µl distilled water as a negative control were added. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid released was removed, and tubes were again weighed to observe the difference in weight after clot stabilization. The obtained difference in weight was expressed as a percentage of lysed clot. In the another Eppendorf tubes second series of experiments, simultaneous addition of 200 µl of blood and 1000 µl of heparin, incubated at 37°C for 90 minutes.

$$\% \text{ of clot lysis} = \frac{\text{Wt of released clot}}{\text{Clot wt}} \times 100$$

$$= \frac{(W2\text{-}W3)}{(W2\text{-}W1)} \times 100$$

W1=Empty weight of Eppendorf tube,
W2=Weight of Eppendorf tube+clot,
W3=Weight of clot release after addition of plant extract.

***In vitro* antiproteinase activity**

The antiproteinase activity was performed according to Oyedepo and Femurewa [10] with minor modification. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (200-1000 µg/ml). The mixture was incubated for 20 minutes. After incubation 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed as triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS

Qualitative analysis of phytocompounds

Phytochemical screening of aqueous extracts of different part of *W. trilobata* (L.) results was shown in Table 1. In this study, the higher concentration of alkaloids, carbohydrates, proteins, tannins, phenols, saponins, quinines and terpenoids observed in leaf, stem, and flower of *W. trilobata*. Whereas of glycosides and phlobatannins were absent in all the three parts of plant. Flavonoids, coumarins, and anthraquinones were present only in aqueous extract of leaf of *W. trilobata*.

***In vitro* thrombolytic activity**

In this study, aqueous extract of leaf, stem, and flower of *W. trilobata* was screened against thrombus, and the results were shown in Tables 2-4 and Fig. 1. 1 ml of heparin as a positive control (1000 IU/ml) added to clots and consequent incubation for 90 minutes at 37°C, it showed 70.00% lysis of clot. On the distilled water was treated as negative control which revealed as a negligible percentage of lysis of clot (6.25%). The aqueous extract of leaf of *W. trilobata* exhibited a higher degree of thrombolytic activity at all the concentration. The minimum of clot lysis was seen in the aqueous extract of stem of *W. trilobata.*

***In vitro* antiproteinase activity**

The aqueous extracts of *W. trilobata* showed significant antiproteinase activity at different concentration (200-1000 µg/ml), and the results were showed in Table 5 and Fig. 2. The maximum inhibition of proteinase activity (48±4.34) was revealed in the aqueous extracts

Table 1: Screening of bioactive phytocompound in aqueous extract of *W. trilobata*

Name of the phytocompounds	Aqueous extracts		
	Leaf	Stem	Flower
Alkaloids	+++	+++	+++
Carbohydrates	+++	+++	+++
Glycosides	-	-	-
Proteins	+	+	+
Steroids	+	+	+++
Tannins	+++	++	+++
Phenols	+++	+++	+++
Flavonoids	+	-	-
Coumarins	+	-	-
Saponins	+++	+++	+++
Quinines	+++	++	+++
Terpenoids	++	++	+++
Phlobatannins	-	-	-
Anthraquinones	++	+	+++

+++ (highly); ++ (moderate); + (mild); - (absent); + (present).
W. trilobata: Wedelia trilobata

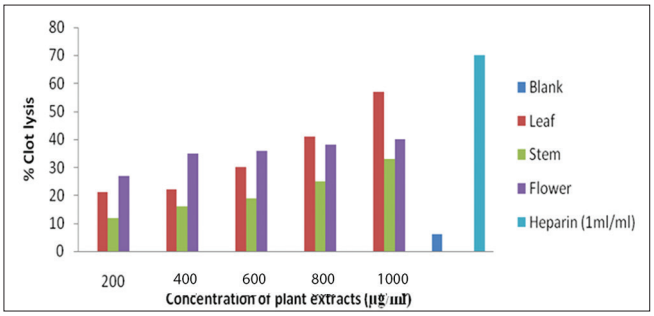

Fig. 1: *In vitro* thrombolytic activity of aqueous extracts of leaf, stem, and flower of *Wedelia trilobata* (L.). Values are expressed as mean±standard error (n=3)

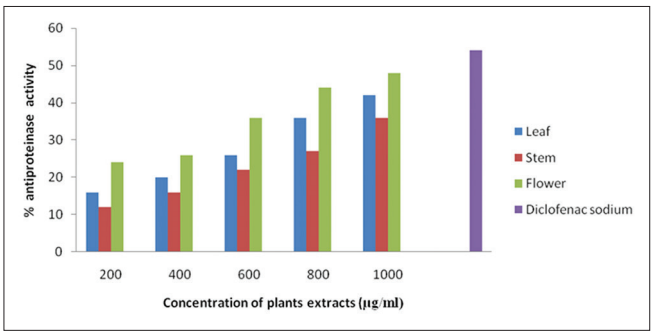

Fig. 2: *In vitro* antiproteinase activity of aqueous extracts of *Wedelia trilobata* (L.). Values are expressed as mean±standard error (n=3)

Table 2: *In vitro* thrombolytic activity of aqueous extract of *W. trilobata* leaf

Concentration of plant extracts (µg/ml)	Weight of empty tube (A) g	Weight of tube with clot (B) g	Weight of clot (C) (B-A) g	Weight of tube with clot after lysis (D) g	Weight of lysis (E) (B-D)	% of clot lysis	Average % of clot lysis
Blank	1.030	1.140	0.110	1.130	0.010	9.09	
200	1.010	1.200	0.190	1.160	0.040	21.05	
400	1.030	1.120	0.090	1.100	0.020	22.22	
600	1.020	1.150	0.130	1.110	0.040	30.76	35.208
800	1.030	1.200	0.170	1.130	0.070	41.17	
1000	1.020	1.210	0.190	1.100	0.110	57.89	
Heparin (1 m1/m1)	1.040	1.180	0.140	1.090	0.090	64.28	

W. trilobata: Wedelia trilobata

Table 3: *In vitro* thrombolytic activity of aqueous extract of *W. trilobata* stem

Concentration of plant extracts (µg/ml)	Weight of empty tube (A) g	Weight of tube with clot (B) g	Weight of clot (C) (B-A) g	Weight of tube with clot after lysis (D) g	Weight of lysis (E) (B-D)	% of clot lysis	Average % of clot lysis
Blank	1.010	1.170	0.160	1.160	0.010	6.25	
200	1.000	1.160	0.160	1.140	0.020	12.50	
400	1.030	1.090	0.060	1.080	0.010	16.66	
600	1.030	1.140	0.110	1.120	0.020	18.18	24.560
800	1.010	1.090	0.080	1.070	0.020	25.00	
1000	1.010	1.100	0.090	1.070	0.030	33.33	
Heparin (1 m1/ml)	1.020	1.120	0.100	1.060	0.060	60.00	

W. trilobata: Wedelia trilobata

Table 4: *In vitro* thrombolytic activity of aqueous extract of *W. trilobata* flower

Concentration of plant extracts (µg/ml)	Weight of empty tube (A) g	Weight of tube with clot (B) g	Weight of clot (C) (B-C) g	Weight of tube with clot after lysis (D) g	Weight of lysis (E) (B-D)	% of clot lysis	Average % of clot lysis
Blank	1.020	1.120	0.100	1.130	0.010	8.33	
200	1.020	1.130	0.110	1.100	0.030	27.27	
400	1.010	1.180	0.170	1.120	0.060	35.29	
600	1.030	1.140	0.110	1.100	0.040	36.36	36.392
800	1.010	1.170	0.160	1.120	0.060	37.50	
1000	1.030	1.130	0.100	1.090	0.040	40.00	
Heparin (1 ml/ml)	1.020	1.120	0.100	1.050	0.070	70.00	

W. trilobata: Wedelia trilobata

Table 5: *In vitro* antiproteinase activity of aqueous extracts of *W. trilobata*

Concentration of plant extracts (µg/ml)	% antiproteinase activity			Diclofenac sodium (1 mg/ml)
	Leaf	Stem	Flower	
200	16±4.33	12±3.88	24±4.12	-
400	20±3.72	16±4.33	26±3.33	-
600	26±3.33	22±4.02	36±4.33	-
800	36±3.77	27±4.21	44±3.90	-
1000	42±4.92	36±4.21	48±4.34	53±4.34

Values are expressed as mean±SE (n=3). SE: Standard error, *W. trilobata: Wedelia trilobata*

of *W. trilobata* flower at the concentration of 1000 µg/ml. Which was compared with leaf and stem of *W. trilobata* and diclofenac sodium (1 mg/ml). The minimum antiproteinase activity (12±3.88) exhibited in stem extract of *W. trilobata* at the concentration 200 µg/ml. In this study, the membrane stabilization was improved on increasing concentration of plant extracts.

DISCUSSION

The phytochemicals access is very significant aspects in pharmacognostic evaluation of medicinal plants [11]. The researcher reported phytocompounds possess antioxidant, antimicrobial, and anti-inflammatory properties and use in the therapy of various infection diseases, etc. [12]. Plants are an important source for the development of potential new chemotherapeutic drugs and its use as a health treatment in folklore medicine [13]. The bioactive of plant extracts is qualified to phytochemical constituents alkaloids isolated and characterized from *Alstonia rupestris* with cytotoxic, antibacterial, and antifungal activities [14] also antioxidant properties [15]. These secondary metabolites are possess various pharmacological activities are hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, and antileprosy [16]. Nisreen and Anil [17] reported the phytochemical analyses of flower extracts of *W. trilobata*. Similarly, in this study, the various pharmacological active phytoconstituents observed in aqueous extracts of leaf, stem, and flower of *W. trilobata.*

Atherothrombotic diseases like myocardial or cerebral infarction occur due to the development of thrombus that causes interruption in the passage of vessels [18]. At present, various thrombolytic agents in practice are being used to dissolve the clots that have already formed in the blood vessels; but these drugs have certain restrictions and can lead to possible fatal consequences in some cases. A number of researchers reported the plant and natural products possess thrombolytic activity (anticoagulant and antiplatelet) [19].

A natural fibrinolytic agent is plasmin, lyses clot by disrupting the fibrinogen and fibrin contained in a clot. Again, cell surface bound plasminogen is easily activated to plasmin, which could lead to fibrinolysis [20]. Streptokinase forms a complex with plasminogen, which is converted plasminogen to plasmin [21]. Scientists have discovered several thrombolytic drugs from various sources [9]. Those thrombolytic drugs more site specific and effective [22]. Unfortunately, side effects related to these drugs such as bleeding and embolism [23]. In past two decades toward the investigation, finding, design, and improvement of natural products with anticoagulant [24], antithrombotic [25], and thrombolytic activity of the plants [26]. Few plant extracts and their products having fibrinolytic activity are identified, which includes *Flammulina velutipes* [27], and *Ganoderma lucidum* [28], ginger (*Zingiber officinale*) [29], and garlic (*Allium sativum*) [30].

Proteinases have been associated in arthritic reactions. Neutrophils are known to be a source of proteinase, which carries in their lysosomal granules many serine proteinases. It was earlier reported that leukocytes proteinase play a central role in the improvement of tissue damage through in inflammatory reactions and important level of defense was provided by proteinase inhibitors [31]. Plant phenolic and flavonoids compounds possess potent anti-inflammatory activity [32,33]. Tannins, saponins, and terpenoids were observed plant extracts may possess potent and anti-inflammatory properties [34-36]. In this study, the maximum antiproteinase activity showed in aqueous extracts of *W. trilobata* flower, it may be due to the presence of flavonoids and related polyphenols compounds as contributed significant to antiproteinase activities against proteinase action [37].

CONCLUSION

A medicinal plant is contain bioactive substances that can be used for a therapeutic purpose which are precursors for the synthesis of useful drugs. In this study, *in vitro* thrombolytic and antiproteinase activities of different parts of *W. trilobata.* Phytochemical analysis of aqueous extracts of leaf, stem, and flower of *W. trilobata* showed carbohydrates, protein, phenols, tannins, saponins, alkaloids, terpenoids, and quinines in all three parts of aqueous extracts. The maximum dissolution of blood clot (fibrinolysis) was observed in leaf extracts *W. trilobata*. The highest degree of proteinase inhibitory activity was showed in flower of *W. trilobata.* Hence, the aqueous extracts of a different part of *W. trilobata* revealed that significant thrombolytic and antiproteinase activities. In further, to isolate the phytocompounds, from this plant for synthesis of the effective drug in thrombolytic and anti-inflammatory therapy.

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