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Der Pharmacia Lettre, 2016, 8 (6):339-341
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***In vitro* thrombolytic activity of *Hemigraphis alternata* (Burm.f.) T. Anderson leaf extract**

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ABSTRACT

Plants are being extensively explored for harbouring medicinal properties. They are one of the major sources for drug discovery and development. Nature had been known as stockyard of medicinal agents since the time immemorial. Herbal products are extensively perceived as safe because they are natural having less or no side effects. In this aspect, *Hemigraphis alternata* (Burm.f.) T. Anderson was shown to possess many medicinal properties. The *in vitro* thrombolytic activity of *H. alternata* leaf extract was evaluated. In the hot and cold water leaf extracts of *H. alternata* the *in vitro* thrombolytic activity was recorded as $96 \pm 0.70\%$ and $80 \pm 0.97\%$, respectively. Highest thrombolytic activity was found to be preserved by the hot water extract which could be recommended for curing human ailments.

Key words: *Hemigraphis alternata*, clot lysis, thrombolytic activity

INTRODUCTION

Medicinal plants have been a valuable source of natural active constituents that products for maintain human health and treatment of many human disease [1]. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopoeia as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens [2]. *H. alternata* (Acanthaceae), an exotic plant adapted to India, is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. Literally, *Hemigraphis* means 'half writing' because the filament of the outer stamen bear brushes [3].

Thrombolysis is a complex mechanism which interacts with clot components and surrounding plasma. In this interaction, plasmin, plasminogen, plasminogen activator and fibrin are involved [4]. The thrombolytic activity of plasma is physiologically very important [5]. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Due to shortcoming of accessible thrombolytic drugs, studies are underway to design new improved recombinants variants regimen [6]. Aspirin and Heparin are significantly effective for activation of lysis and prevention of reocclusion [7]. The selective antiplatelet agents and thrombin inhibitor are most potent though safety is yet a main concern. The new studies and investigations in this area will give new imminent that encourage the advancement of the ideal thrombolytic therapy.

MATERIALS AND METHODS

Sample collection and processing

Freshly collected *H. alternata* leaves were washed in running tap water for 3 min. Then the plant parts were surface sterilized using 1% mercuric chloride solution under strict aseptic conditions. Finally they were rinsed with sterile distilled water thoroughly to remove mercuric chloride residues. Excess moisture was removed from the sterilized leaves and flower. Then they were subjected to hot water and cold water extraction.

Hot water extraction

About 10 g fresh leaves of *H. alternata* were boiled in 100 mL distilled water with constant stirring for 30 min. The solution was then allowed to cool to room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whatman's No. 1 filter paper under strict aseptic conditions. The filtrate was collected in fresh sterilized glass tubes and stored at 4 °C until use [8].

Cold Water Extraction

About 10 g fresh leaves of *H. alternata* was macerated in pestle and mortar with 100 ml distilled water at room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 min. The supernatant was again filtered using Whatman's No. 1 filter paper under strict aseptic conditions and the filtrate was collected in fresh sterilized glass tubes and stored at 4 °C until use [8].

In vitro thrombolytic activity

About 3 mL of fresh blood was drawn from volunteers (n=4) and immediately distributed in the 5 different pre weighed sterile microfuge tubes (0.5 mL/tube). The tubes were incubated at 37 °C for 45 min. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). About 100 µL of flower and leaf extract (hot and cold) was added to the clot tube at different concentrations. About 100 µL of distilled water in a clot tube serves as negative control and aspirin was used as standard drug. All the tubes were incubated at 37 °C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption [9].

$$\% \text{ of clot lysis} = (\text{weight of released clot} / \text{clot weight}) \times 100$$

RESULTS AND DISCUSSION

The use of herbal for treatment of disease has been in practice since ancient times. Herbal medicines are considered safer due to their natural activity [10]. It has been reported from studies that herbal products showing their thrombolytic activity significantly [11]. With the advancement in phytochemistry and identification of new plant compounds having significant efficacy against certain diseases, it has been proved by research conducted on herbal medicines. The anti thrombotic activity of herbs and their natural compound has reported previously [12]. In the present study, the thrombolytic activity of aqueous extracts of *H. alternata* leaf extracts (hot and cold water) was carried out using a simple and rapid *in vitro* clot lysis model. In the hot and cold water leaf extracts of *H. alternata* the *in vitro* thrombolytic activity was recorded as 96±0.70% and 80±0.97%, respectively (table 1).

Table 1: Thrombolytic activity of plant extract

S.No.	Plant extracts	Thrombolytic activity (%)
1	<i>H. alternata</i> leaf extracts (hot water)	96±0.70
2	<i>H. alternata</i> leaf extracts (cold water)	80±0.97

CONCLUSION

Based on above ideas *H. alternata* possessed medicinal properties and so it can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address unmet therapeutic needs such screening of warriors natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. From the results of the study it is concluded that aqueous hot water *H. alternata* leaf extract showed the highest percentage of clot lysis, which suggests that this extract could be used as effective clot lysis agent.

Acknowledgement

We acknowledge DBT-STAR college scheme, Department of Biotechnology (DBT), Government of India, New Delhi for their financial support and encouraging research in undergraduate level. Authors are thankful to Secretary, Principal and Department of Biotechnology of Kongunadu Arts and Science College for providing facilities and encouragement to carry out this research work.

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