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A STUDY ON THE *IN-VITRO* THROMBOLYTIC ACTIVITY OF *OSCILLATORIA SPECIES*

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ABSTRACT

Thrombolytic diseases such as myocardial, cerebral infarctions are serious consequences of the thrombus formed in blood vessels which are the most common causes of death worldwide. Tissue plasminogen activator (t-PA), Streptokinase (SK), Urokinase (UK), are the thrombolytic agents used to dissolve the already formed clots in the blood vessels. These chemical agents used for thrombolytic activity causes side effects like allergic reactions, bleeding, hypotension, low-grade fever, rash, hypersensitivity etc. Alternative to chemical agents is natural agents which will prevent these side effects. In view of this, the current study is aimed to identify the thrombolytic activity of the Algae species *Oscillatoria* (cyanobacteria). Bioactive active compounds were extracted from the algae using methanol as solvent. The methanolic extract is tested for its thrombolytic activity by RBC lysis method. The *in-vitro* thrombolytic activity study revealed that the methanolic extract of *Oscillatoria* showed significant clot lysis when compared with the positive control (streptokinase) and negative control (ethanol). The mean clot lysis % difference was significant. From the *in-vitro* study, we demonstrate that the algae extract has very good clot lysis activity and it could be considered as potential sources of natural thrombolytic agents.

KEYWORDS: *Oscillatoria* species, cyanobacteria, clot lysis, thrombolytic activity, thrombolytic agents.

INTRODUCTION

The pathologic process of a blood clot forming inside a blood artery and restricting blood flow via the circulatory system is called thrombosis. Thrombosis can lead to embolism, ischemia, heart attacks, strokes, and other complications (Monie and DeLoughery, 2017). Thrombotic disorders have become one of the serious problems as a result of population expansion, ageing, and changing lifestyles. It is a significant contributor to morbidity and death (Roth *et al.*, 2020). To treat thrombotic diseases, blood clots must dissolve quickly, and the blood flow should be restored. The most promising therapeutic drug for the treatment of thrombosis is thought to be fibrinolytic enzymes (Kotb E, 2013). These enzymes are divided into groups based on how they work like plasminogen activators, such as the tissue-type plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), streptokinase, plasmin-like, and lumbrokinase fibrinolytic enzymes (Peng *et al.*, 2005). Also, antiplatelet medicines are those that interfere with platelet function and are utilised in the treatment of thrombotic illnesses since platelets are essential for the onset of thrombosis (Bhatt DL, 2009; Ren *et al.*, 2010). Numerous antiplatelet medications are available for use in clinical settings. Although useful, current medications including aspirin, ticagrelor, clopidogrel, apixaban, and warfarin have undesirable toxicological side effects include prolonged bleeding times, purpura, thrombocytopenia, and gastrointestinal ulcers. Suppression of the immune system is the major side effect of chemotherapeutic agents used clinically to treat thrombosis (Mega and Simon, 2015). The high cost and undesirable side effects of these chemical agents has led researchers to search for cheaper and safer thrombolytic drugs (Lu *et al.*, 2010). Since ancient period, herbal products have been regarded as being less dangerous, and certain research indicate that they can have significant thrombolytic action (Amin *et al.*, 2015; Islam *et al.*, 2018).

The primary source for developing new drugs is natural ingredients. Many plants and microbes are very appealing as a natural source of bioactive compounds (Zahra *et al.*, 2020). Due to the large number of bioactive chemicals with diverse biological functions that have been found in blue-green algae, they have emerged as a key target for the pharmaceutical and biotechnology sectors (Farrokh *et al.*, 2019). Blue-green algae, commonly known as cyanobacteria, are prokaryotes that can flourish in a variety of environmental circumstances and are found worldwide in aquatic and terrestrial settings (Chorus and Bartram, 1999). One of the most significant orders in blue-green algae is *Oscillatoriaceae*. The *Oscillatoriaceae* order contains more than 15 species, including *Spirulina*, *Oscillatoria*, *Phormidium*, and

Lyngbya. More than 300 chemicals belonging to the *Oscillatoriaceae* family have been documented to be produced by these species, covering all of the biological functions (Levasseur *et al.*, 2020). Numerous pharmacological and nutraceutical compounds, including those with antibacterial, antifungal, antioxidant, antialgal, antiviral, anticancer, and anti-inflammatory properties, are produced by *Oscillatoria* species (Sultan *et al.*, 2016). These bioactive substances included terpenoids, N-glycosides, phenolic compounds, alkaloids, fatty acids, pigments and their derivatives, linear and cyclic peptides, and pigments (Demay et al., 2019; Mu *et al.*, 2019). A very few studies have examined the potential application of algal enzymes in fibrinolytic therapy, ushering in a new era of biological exploration of the role of algal enzymes in thrombolytic activity (Diwan *et al.*, 2021). In view of this, the present study is formulated to identify the thrombolytic activity of the Algae species *Oscillatoria* (Cyanobacteria). Hence, the current study aimed at clot lysis activity of methanolic extract of *Oscillatoria sp.* under *in vitro* condition.

MATERIALS AND METHODS

Sample (*Oscillatoria sp.*) collection and extraction

The *Oscillatoria species* culture was collected from Genolites Lab, Coimbatore. The culture medium used for cultivation was BG-11. The biomass of *Oscillatoria s*p., which had been growing for 21 days, was harvested at stationary phase by centrifuging it at 4,000 g for 15 minutes, discarding the aqueous phase, and then drying the pellets at 45 °C until they reached a constant weight before extraction. Then, *Oscillatoria* dry weight was extracted by methanol as solvent at 40 °C by immersing the *Oscillatoria* powder in flask and using an ultrasonication method for 20 min; then, the extract was incubated at 37°C with constant shaking for 1 day at 100 rpm. The extract is centrifuged at 4500 rpm for 10 min and supernatant was collected. The methanolic extract was concentrated in a rotary evaporator at 35°C until it was completely dry. The dried extract was kept in pre-weighed Eppendorf tubes with labels, and kept at 4°C for preservation (Modified method of Chauhan and Johnson, 2010).

In vitro Clot lysis activity of *Oscillatoria* ethanolic extract

According to the approaches recommended by Sweta *et al.* (2007), Fatema *et al.* (2017), and Alawa *et al.* (2018) with relevant modifications for the current study, an *in vitro* experiment to measure thrombolytic activity was planned. The goat blood collected from the local slaughter house were divided into five 1.5 ml microfuge and incubated at 37 °C for three

hours to induce clotting. The serum was then carefully removed, and the weight of the clot was determined. From the 100 mg/ml stock solution of the methanolic extract of *Oscillatoria* species, four sequential concentrations were prepared. 100 µl of the methanolic extract from each concentration was added with the use of a micropipette into the appropriate separated microfuge that was in contact with the blood clot surface. In each individual microfuge, streptokinase and distilled water were used with blood clot which were serving as the corresponding positive and negative controls. The experiment was carried out in triplicates. The set of experiments was incubated for two hours at 37 °C and the *in-vitro* thrombolytic activity was monitored. After incubation, the liquid released was drained, and any remaining clot was rinsed with sterile distilled water and dried to eliminate moisture. The percentage of thrombolytic activity of the extract in comparison to the standard was calculated using the final weights of the microfuge using the following formulae.

% Clot lysis = (Weight of Clot after incubation / Weight of Clot before incubation) x 100

RESULTS AND DISCUSSION

In vitro thrombolytic activity of ethanolic extract of *Oscillatorisa sp.* considered in this study demonstrate the promising results in terms of new therapeutic substance development. There were 4 concentrations of the extract used, to determine the rate of thrombus lysis. The thrombolytic activity of the extract is shown in Table 1 by comparison with streptokinase as standard.

Table 1: *In vitro* clot lysis activity of ethanolic extract of *Oscillatoria sp.*

S.NO.	Sample (100 µl)	Description	Concentration	Clot lysis %
1	C	Negative control with distilled water	Not Applicable	1.9 %
2	S	Positive control with Streptokinase	0.1 mg	93.5 %
3	T1	Concentration 1	1 mg	18.7 %
4	T2	Concentration 2	2.5 mg	36.2 %
5	T3	Concentration 3	5 mg	67.5 %
6	T4	Concentration 4	10 mg	82.4 %

The *in-vitro* thrombolytic activity study of *Oscillatoria* algae revealed that the methanolic extract of the algae shows good clot lysis when compared with a negative control (distilled water) and the mean clot lysis percentage was significant when compared to standard streptokinase. At the concentration of 10 mg/ 100 µl, clot lysis percentage was found to be 82.4%, which was reduced to 67.5%, 36.2%, 18.7% at the concentrations 5 mg/ 100 µl, 2.5 mg/ 100 µl, 1 mg/ 100 µl respectively. In our study, the positive control streptokinase showed

93.5%, which produces a thrombolytic activity in range from 80 to 95 % at the concentration 0.1 mg (Zaman *et al.*, 2015; Ashrafudoulla *et al.*, 2016).

The *in-vitro* thrombolytic study revealed that methanolic extract of *Wedelia chinensis* Osbeck., *Emilia sonchifolia (L.)* DC., *Eclipta alba* (L) Hassk and *Spilanthes paniculate* wall. Showed 24.48%, 28.71%, 15.19% and 42.77% clot was lysed. The percentage % was calculated treating clot with these two extract and appropriate control (Fatema *et al.*, 2017). Hence, the results of the current study suggest that the blood clot dissolution by the methanolic extract of *Oscillatoria sp.* is considerable. Even though the thrombolytic activity in the current investigation was reported at greater concentrations when compared to streptokinase, obtaining methanolic extracts of algae material is simple and affordable.

CONCLUSION

From the *in-vitro* thrombolytic study, it could be suggested that the methanolic extract of *Oscillatoria* species can be considered as potential sources of natural thrombolytic agents. This is only the preliminary study and the extract should be investigated further to exploit their phytochemical compounds and /or enzymes responsible for the activity. Also, substantial and rigorous research on their physiological, cytotoxic, and genotoxic studies, compatibility and pharmacological tests should be conducted *in vivo*. The thrombolytic properties of the methanolic extract of the algae *Oscillatoria Sp*. might be used to build new therapeutic technologies and pharmacological products that could be easy to use, inexpensive, and possibly secure owing to their native environment.

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