THROMBOLYTIC ACTIVITY AND ANTIMICROBIAL PROPERTIES OF SCF EXTRACT OF *CURCUMA AERUGINOSA* Roxb.

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ABSTRACT

The in vitro techniques and the biological investigations of microbiological and the phytochemical tests, which includes antimicrobial and thrombolytic study of SCF extract of *Curcuma aeruginosa*. This plant is used in the traditional medicinal system for cure of a variety of diseases. The plant has a characteristic rhizome of bluish-black color with pungent smell and hot bitter taste. The rhizome is traditionally used in the treatment of hemorrhoids, leprosy, asthma, cancer, fever, wounds, vomiting, menstrual disorder, anthelmentic, aphrodisiac, gonorrheal discharges and inflammation. Furthermore, the smooth muscle relaxant, anti-tumour and anti-oxidant properties of *C. caesia* rhizome extract had been reported. Due to its high medicinal value, the plant is in great demand in central India. The SCF extract of the plant possess thrombolytic activity, along with streptokinase as a positive control and water as a negative control. Using an in vitro thrombolytic model the extract showed (77.11±2.13%) thrombolytic activity. Through this study it was found that it possesses thrombolytic properties that could lyses blood clots *in vitro*. The antibacterial activity was determined by using the agar diffusion method. The extract of the plant showed moderate activity against Gram positive bacteria *Bacillus subtilis* (13.7±1.53) and gram negative bacteria *Klebsiella pneumoniae* (13.3±1.15). The concentration of the extracted sample was 2mg/20µl was used for antibacterial activity.

Keywords: streptokinase, Thrombolytic activity, SCF extract, antibacterial activity.

Introduction

Plants are considered not only as dietary supplement to living organisms but also conventionally used for treating many health problems and the medicinal value of many plants still remains unexplored. Investigations of plants are carried out to find new drugs or templates for the development of new remedial agents [1].

Traditional medicine in developing countries uses a wide variety of natural products in the treatment of some common deceases. Blood clot formation, thrombosis, has been a severe problem of the blood circulation system. Thrombus or embolus obstructs the blood flow by blocking the blood vessel therefore depriving blood and oxygen supply to tissue, leading to tissue necrosis. Atherothrombotic diseases such as acute myocardial or cerebral infarction and stroke are serious consequences of the thrombus formed in blood vessels. Usually thrombolytic agents such as streptokinase (SK),urokinase (UK), or tissue-plasminogen activator (t-PA) [2]-[4], are used to dissolve the formed clots in the vessels, however, these drugs have certain limitations which cause severe and sometime fatal disorders including systemic fibrinolysis, anaphylactic reaction, and bleeding tendency [5]-[7].Traditional herbs have been used since ancient times to treat many diseases. Herbs are often known as safe because they are natural products. Previous studies have shown that many herbs possessed antithrombotic activity [8]-[11].Plants produce wide array of bioactive principles and is important for the human body to eliminate the toxicity constitute a rich source of medicines. In many developing countries, traditional medicine is one of the primary health care systems [12, 13]. For the past two decades, there has been an increasing interest in the investigation

of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (14). The plant sap can act against microorganisms by preventing the growth of microbial colony(15). Medicinal plants have a remarkable capasity to produce a wide variety of bioactive secondary metabolites, like alkaloids, terpenoids, glycosides, saponins, flavonoids, steroids, tannins, quinones and coumarins(16). These biomolecules are the source of plant-derived antimicrobial substances (17). Some natural products are highly efficient in the treatment of bacterial infections (18). Which are obtained from a wide variety of natural resources like plant leaves, bark, berries, flowers and roots (19). *Curcuma aeruginosa* Roxb. belonging to family Zingiberaceae is a versatile medicinal plant documented in Ayurveda used as medicine for the treatment of a variety of diseases and disorders.The present study focused on to evaluate the thrombolytic and antibacterial effects of rhizome SCF extract.

Materials and methods

In vitro thrombolytic activity

Streptokinase (SK): Commercially available lyophilized Streptokinase vial of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for in vitro thrombolysis(20).

Blood sample: Blood (n=5) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic activity: The thrombolytic activity of extracts was evaluated by the method developed by (21) and slightly modified by (22) using streptokinase (SK) as the standard. Venous blood was drawn from healthy volunteers (n = 5) and transferred in different pre-weighed sterile alpine tube and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of the tube alone). Each alpine tube containing clot was properly labeled and 100 µl of *Curcuma aeruginosa* rhizome extracts was added to the tubes separately. As a positive control, 100 µl of Streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

Antimicrobial activity

Microorganisms used for the test: The present study was carried out with the bacterial strains procured from IMTECH, Chandigarh, India. The bacterial strains used for antibacterial screening were *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-1687), *Klebsiella Pneumoniae* (MTCC- 3384) and *Proteus vulgaris* (MTCC-744) The bacterial strains were maintained on nutrient agar slants, sub cultured regularly and stored at 4^{o}C for further use.

Inoculum preparation: One loop full of overnight grown bacterial culture was inoculated in 25 ml nutrient broth at 37°C on a rotary shaker incubator for 16-18 h. The inoculum size of each bacterial strains were standardized by adjusting the optical density of the culture broth to a turbidity corresponding to 0.08 at 620 nm using a spectrophotometer which is equivalent to 10^8 cfu/ml (23)

Assessment of antibacterial activity: The antibacterial activity of the crude extracts was determined by the agar-well diffusion method (24). 200 µl of the standardized cell suspension were spread on Nutrient Agar (Hi-media) plate using a sterile swab. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. The crude extract was introduced into the well at a concentration of 2mg/20µl, allowed to stand at room temperature for about 1 h as a period of pre- incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 37°C for 24 h. Controls were also set up in parallel and the effects were compared with streptomycin at a concentration of 10µg/20µl. The plates were observed for the zone of inhibition after 24 h. The experiment was conducted in triplicates and the results are expressed as mean ± SE.

Results and Discussion

As a part of discovery of cardio-protective drugs from natural sources the extractives of *Curcuma aeruginosa* rhizome were assessed for thrombolytic activity and the results are presented in Table 1, Figure 1. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 89.56±1.13% lysis of clot. At the same time, distilled water was treated as negative control which exhibited negligible

lysis of clot (7.2±0.89%). In this study, the rhizome extract of *Curcuma aeruginosa* exhibited highest thrombolytic activity.

Anti-Bacterial Activity: The antibacterial activity of the SCF extract of *Curcuma aeruginosa* Roxb, was analyzed against both Gram-positive and Gram-negative bacteria, the results of which are presented in Figure 2 and Table 2. From the results it is evident that SCF extract has showed anti-bacterial activity. In Gram Positive bacteria Bacillus subtilis showed maximum zone of inhibition 13.7 mm whereas Gram negative bacteria Klebsiella showed 1.3 mm of zone of inhibition. (77.11±2.13%)

CONCLUSION: The present investigation was carried out to study the thrombolytic and antibacterial potentiality in the SCF extracts of *Curcuma aeruginosa* against two gram positive and three gram negative bacteria. The thrombolytic comparison of positive control with negative control indicated that the blood clot did not dissolve when distilled water was added to the clot. However, in vivo clot dissolving properties and active components for clotlysis are yet to be discovered. Once found could be incorporated as a thrombolytic agent for the improvement of patients suffering from Atherothrombotic diseases. The above findings would be a primary platform to explore local potential medicinal plants possessing antimicrobial efficacy and their further exploration proves to be the bedrock for future medicine.

Table 1. Thrombolytic activity of SCF extract of *Curcuma aeruginosa*

Sample	Thrombolytic activity (% of lysis)
Control (Streptokinase)	**89.56±1.13**

Water	**7.2±0.89**
SCF	**77.11±2.13**

Figure: 1

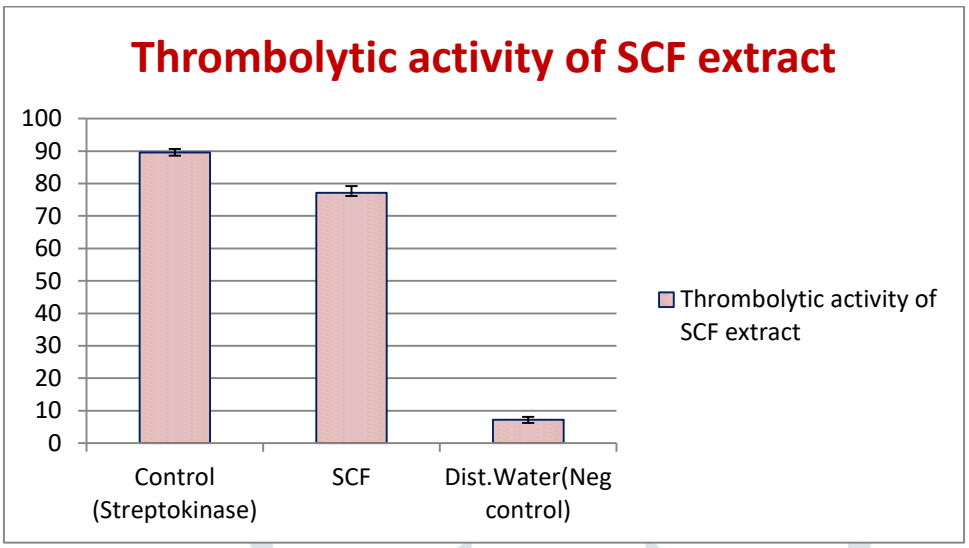
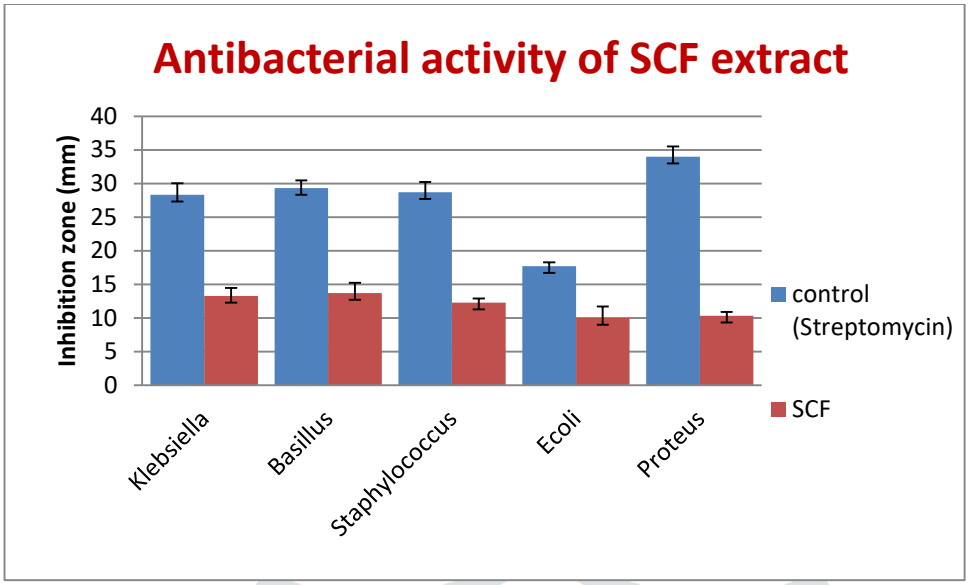


Table:2 Antibacterial activity of SCF extract of *Curcuma aeruginosa*

Bacterial species	Klebsiella pneumoniae	Basillus subtilis	Staphylococcus aureus	Ecoli	Proteus vulgaris
SCF Extract	13.3±1.15	13.7±1.53	12.3±0.58	10±1.73	10.33±0.57
Control (Streptomycin)	28.3±1.73	29.3±1.15	28.7±1.52	17.7±0.58	34±1.53

Figure: 2



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