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Assessment of Thrombolytic Potential using Ethanolic Leaf Extract of *Buchanania lazan* and *Holoptelea integrifolia.*

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ABSTRACT

The study was carried out to investigate into the thrombolytic potential of ethanolic leaf extract of two medicinally important trees- *Buchanania lanzan* and *Holoptelea integrifolia*. Different concentrations of the crude extracts (3, 5, 7, and 9 mg/ml) were used for the study following standard protocol. The results showed mild clot dissolving ability, i.e. 11.16±0.723%, 8.65±1.058%, 11.40±0.626%, 12.82±0.747% in *Buchanania lanzan* and 10.26±1.79%, 16.82±0.605%, 8.37±0.578%, 5.08±0.570% in *Holoptelea integrifolia* at 3, 5, 7, and 9 mg/ml concentrations respectively. The total phenol content of Buchanania lanzan and Holoptelea integrifolia are 53.06 µg/ml and 121.89 µg/ml with the characteristic wave number of phenolic OH in FT-IR spectra 3329.19 cm^{-1} and 3332.99 cm^{-1} respectively. The results suggest that the ethanolic leaf extract of *Buchanania lazan* and *Holoptelea integrifolia* are endowed with anticoagulant activity.

Keywords: *Buchanania lazan*, *Holoptelea integrifolia*, Ethanolic extract, Thrombolytic activity

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INTODUCTION

Thrombolysis also known as thrombolytic therapy, is used to treatment that could cause serious and possibly life threating damage if they are not removed i.e to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs, also it can prevent heart attack or brain stroke. Thrombolysis may involve the injection of clot-busting drugs through an intravenous (IV) line or through a long catheter that delivers drugs directly to the site of the blockage. It also may involve the use of a long catheter with a mechanical device attached to the tip that either removes the clot or physically breaks it up.

Any substance that, in vivo or in vitro, suppresses, delays, or nullifies coagulation of the blood is termed as anticoagulant agent which includes heparin and the coumarin compound [11, 4, 7, 9] those are capable of disintegrating thrombin that have already formed i.e thrombolytic, such as streptokinase and urokinase. There as another group of anticoagulant i.e. antiplatelet agent which prevent the clumping together of platelets, a primary step in the formation of thrombin specially in cerebrovascular system [8, 17].

Several plants used for the treatment of thromboembolic diseases in different systems of traditional medicine have shown anticoagulant/antithrombotic activity and such plants claimed in the traditional system still remain to be scientifically investigated. For more than five decades, anticoagulant drugs consisting of heparins, vitamin K antagonists, and their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. Plants may serve as the alternative sources for the development of new anticoagulant agents due to their biological activities [14]. There is compelling scientific evidences demonstrating that the consumption of dietary anticoagulants or phytochemicals with anticoagulant properties can ultimately reduce or eliminate the risks of thromboembolic diseases. Prothrombin time (PT) is measure of the extrinsic coagulation pathway.

Many researchers investigation based on the anticoagulant activity of some plant such as study the anticoagulant activity of red onion extract and garlic oil [2], aqueous and ethanol extracts of leaves and whole plant of four medicinal plants such as Enicostemma littorale, Acheranthus aspera, Tridax procumbens [15], aqueous leaves extract of *Jatropha gossypiifolia* L.[6], methanolic leaf extract of *Artemisia dracunculus* L. [4]seed powder of *Pentaclethra macrophylla* [1] aqueous and ethanolic extract of *Phyllanthus niruri* L. [10].

The aim of the present study was to evaluate the anticoagulant potential of two plant i.e *Buchanania lanzan* and *Holoptelea integrifolia* belongs to the family Anacardiaceae and Ulmaceae respectively. *Buchanania lanzan* possesses antidiabetic, antihyperlipidemic, antioxidant, anti-inflammatory, wound healing, antidiarrheal, antivenom activity and other curative properties [3] and contain a number of secondary metabolites among them coumarins are known to responsible for anticoagulant property [13]. Holoptelea integrifolia is extensively used for various curative properties like antiviral, antimicrobial, antifungal, anti-arthritic, antioxidant, wound healing, anti-helmenthic, anti-diabetic, anti-diarrheal, antiulcer, antitumor, adaptogenic, analgesic, hepatoprotective, larvicidal activities [16].

MARETIAL AND METHODS

Plant material

Fresh leaves of plant specimens i.e *Buchanania lanzan and Holoptelea integrifolia* was collected randomly from their naturally occurring sites, e.g. Shibpur forest beat Durgapur and Golapbag campus of Burdwan University respectively.

The plant was identified and its voucher specimen was kept in the herbarium of Burdwan University (**BURD)** for future reference. The mature leaves were washed thoroughly under running tap water and distilled water, blotted dry and then finally dried by keeping inside hot air oven at 50°C temperature.

Sample preparation

The dried powdered leaves of these plants (10 g) were extracted in 100 ml of 95% ethanol. The resultant ethanolic extracts were filtered through a vacuum filter and the filtrates were defatted using hexane. The ethanolic portions were dried and used for analysis.

Standard drug and blood sample

Streptokinase (SK) To the commercially available lyophilized *Streptokinase vial* of 15, 00,000 I.U., 5 ml 0.9% sodium chloride (NaCl) was added and mixed properly. This solution was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis assay. Whole blood (11 ml) was drawn from healthy human volunteers (*n = 3)* (aged 25-30 years) *without a* history of oral contraceptive or anticoagulant therapy (using a protocol approved by Institutional Ethics Committee). 1 ml of blood was transferred to each of the 11 previously weighed sterilized micro-centrifuge tubes to form clots.

Preparation of test sample

Five different test solutions were used to evaluate the thrombolytic activity of the plant extract. The plant extract was dissolved in distilled water and shaken vigorously on a vortex mixer to prepare different concentrations 3, 5, 7, and 9 mg/ml respectively) of the test sample. The suspension was kept overnight and decanted to remove the soluble supernatant, which was then filtered. 100 µl of the ethanolic preparations of the plant were added to the micro-centrifuge tube containing the clots to check thrombolytic activity.

Anti-thrombotic assay

In vitro clot lysis activity of the plant extracts was carried out. 11 ml of venous blood was drawn from healthy volunteers (*n = 2)* and transferred to different pre weighed sterile micro-centrifuge tube (1 ml/tube). The micro-centrifuged tubes were subjected to incubation at 37°C for 45 minutes. After the formation of clot, serum was completely removed from the tubes (carried out without disturbing the clot formed) and each tube having clot was again weighed to determine the weight of the clot (clot weight = weight of clot containing tube – weight of tube alone). Each micro-centrifuge tube containing clot was properly labeled and 100 µl of the plant extract with various concentrations (3, 5, 7 and 9 mg/ml respectively) was added to the tubes accordingly. As a positive control, 100 µl of streptokinase and as a negative non thrombolytic control, 100 µl of sterilized distilled water were separately added to the control tubes numbered. Then all the tubes were incubated again at 37°C for 90 minutes and observed for clot lysis. After incubation, the obtained fluid was removed from the tubes and they were again weighed to observe the difference in weight after clot disruption.

At last, difference obtained in weight was calculated and the result was expressed as percentage of clot lysis following the underneath equation.

% of clot lysis = (wt. of released clot /clot wt.) × 100

Quantification total phenolic content

Total phenolic contents of the extracts (three replicates per treatment) were assayed using the Foline Ciocalteu reagent and expressed as mg gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50 to 1000 mg/l (R^2= 0.9907). All samples were performed in triplicates.

Analysis of FT IR spectra of the extracts

Fourier transform infrared spectroscopy (FT IR) is one of the most powerful approaches to identify/ characterize the type of chemical bonds (functional groups) present in compounds. The ethanol extracts of these plants were mixed with KBr salt using a morter pestle and compressed into a thin tablets and IR spectra and peacks were recorded on a Perkin Elmer FTIR (model RX1) spectrometer between 4000-400 cm^{-1}. Each of any analysis was twice done for confirmation [11].

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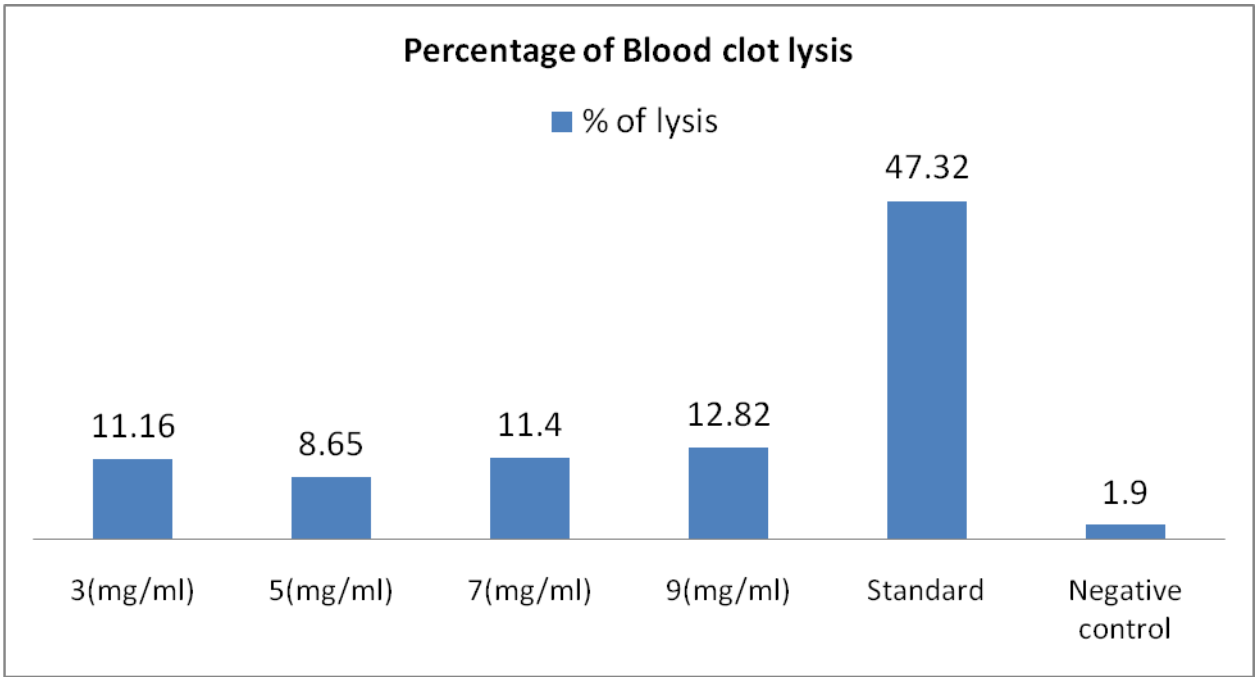
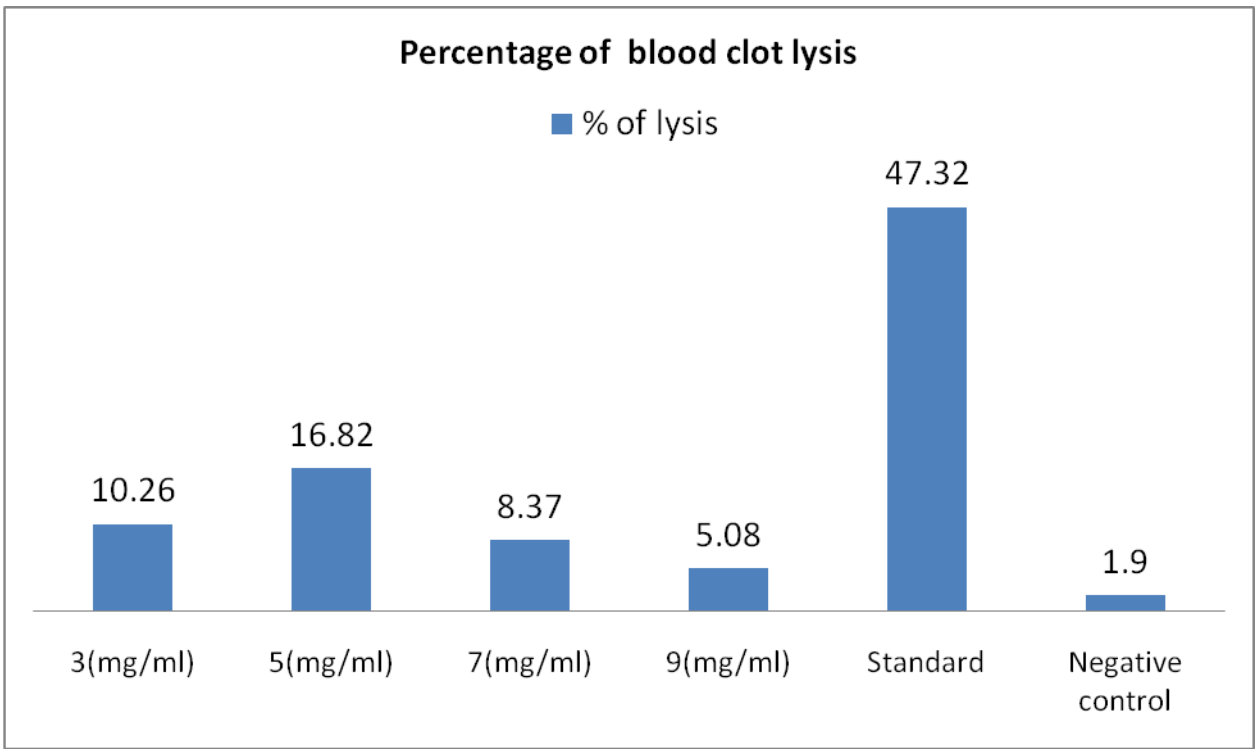
RESULTS AND DISCUSSION



Thrombolytic activity of crude extract of *Buchanania lanzan*, negative control and Streptokinase by clot lysis activity:



Thrombolytic activity of crude extract of *Holoptelea integrifolia*, negative control and Streptokinase by clot lysis activity:

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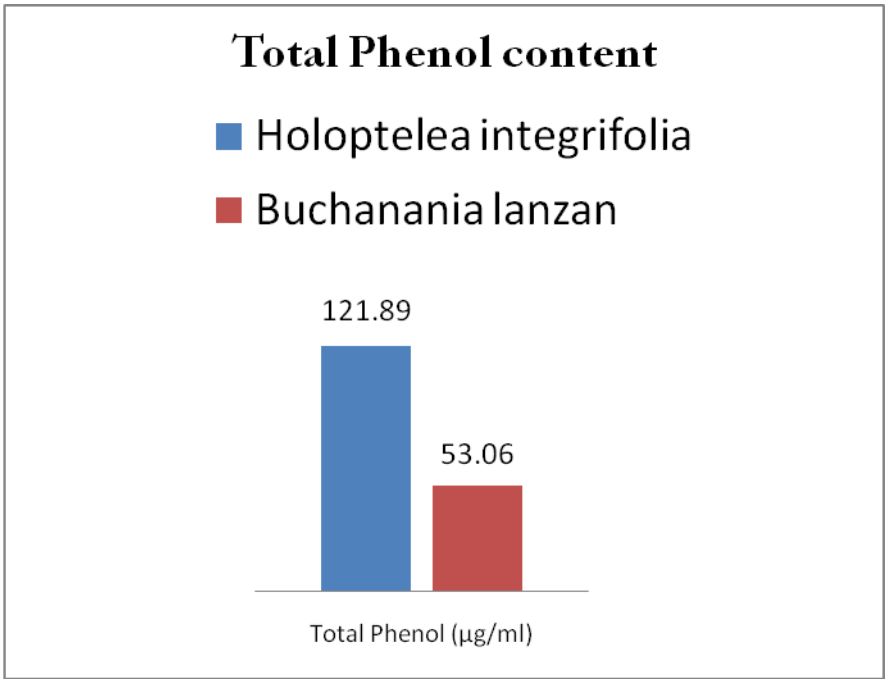
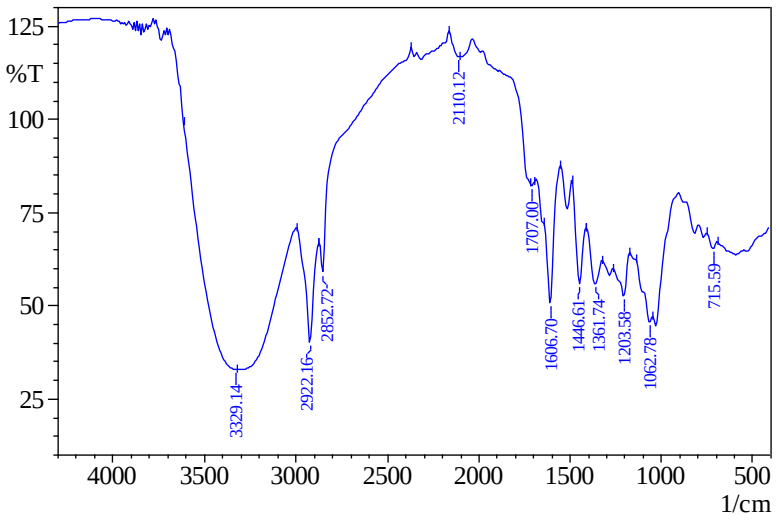
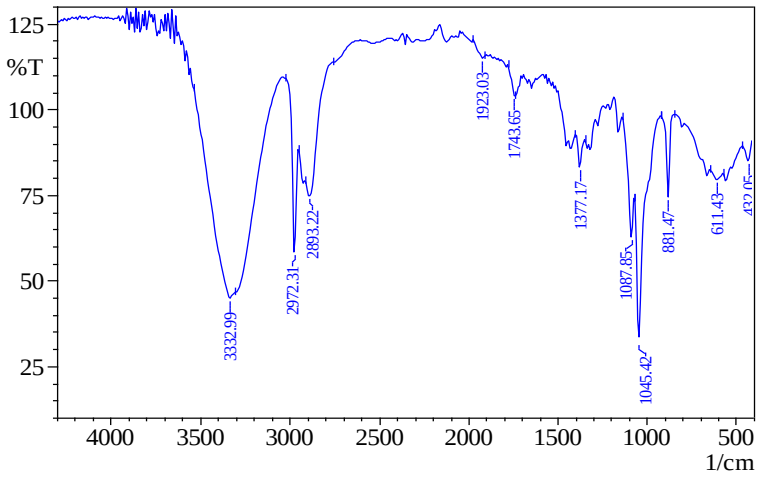




FT IR spectrum of ethanolic extract of *Buchanania lanzan* leaves.



FT IR spectrum of ethanolic extract of *Holoptelea intregrifolia* leaves.

It is evident that the percentage of clot lysis was 47.32% when 100 µl of streptokinase (30,000 I.U.) was used as a positive control, while in case of water (negative control) the percentage of clot lysis was negligible (1.90%).

When clots were treated with 100 µl each of different concentrations (3, 5, 7 & 9 mg/ml respectively) of the test sample *Buchanania lanzan* shows moderate clot lysis activity, i.e., 11.16%, 8.65%, 11.40% and 12.82% and for *Holoptelea integrifolia* it is 10.26%, 16.82%, 8.37% and 5.08% respectively. In *Buchanania lanzan* the percentage of clot lysis drastically decreased in 5 mg/ml concentration and then increased. In *Holopteleea integrifolia* the percentage of clot lysis is highest in 5 mg/ml concentration and then decreased with the increasing concentration of the extract. Phenolic compounds have inhibitory effect on platelet function. Thus encouraging anticoagulant activity and helping in heart diseases [18]. Here the total phenol content of *Holoptelea integrifolia* and *Buchanania lanzan* is 121.89 µg/ml and 53.06 µg/ml respectively. Supporting FT-IR spectrum shows phenolic OH group in 3329.19 cm^{-1} and 3332.99 cm^{-1} for *Buchanania lanzan* and *Holoptelea integrifolia* consequently.

CONCLUSION

The obtained results suggest that the ethanolic leaf extract of *Buchanania lazan* and *Holoptelea integrifolia* possess mild thrombolytic activity; however, in vivo thrombolytic potentiality and active component(s) of the extract responsible for thrombolytic activity requires further analysis.

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