Indian Journal of Pharmacy and Pharmacology 2022;9(1):53–57

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

In-vitro thrombolytic activity study of a polyherbal formulation developed by using extracts of different medicinal plants

Avinash Muduli[1], Susanta Kumar Rout[2], Amiya Kumar Prusty[1,*]

[1]*Dept. of Pharmaceutics, Institute of Pharmacy & Technology, Salipur, Odisha, India*
[2]*Dept. of Science and Technology, Bhubaneswar, Odisha, India*

ARTICLE INFO

Article history:
Received 14-02-2022
Accepted 15-02-2022
Available online 23-03-2022

Keywords:
Thrombolytic activity
Hydroalcoholic Plant extracts
Polyherbal syrup
Streptokinase
Thrombus

ABSTRACT

The present research work was carried out to evaluate the in-Vitro thrombolytic activity of a polyherbal formulation prepared by using extracts of different part of medicinal plants, having thrombolytic activity i.e. bulbs of *Allium* cepa and seeds of *Linum Usitatissimum.* The extracts had shown 38.93 % and 35.65 %, clot lysis activity respectively, that was significant with reference to Streptokinase (64.97 %) and water (1.26%). The formulated poly herbal formulation using these two extracts also had shown good thrombolytic activity. The phytochemical analysis of individul plant extract had shown presence of different phytochemicals in common. That indicates, the in-vitro thrombolytic activity of the plants may be due to the presence of these phytochemicals.

1. Introduction

Thrombus related diseases such as acute myocardial or cerebral infarction occur due to thrombus (blood clot) developed in the circulatory system leading to vascular blockage with serious consequences leading to death also.[1] Therapeutic thrombolytic agents are used to dissolve blood clots by a procedure termed thrombolysis.[2] Streptokinase, urokinase, tissue plasminogen activator (tPA) like alteplase, anistreplase, are commonly used thrombolytic agents that dissolve clots.[3] Heparin and Aspirin are moderately efficient for acceleration of lysis and prevention of reocclusion. Selective third generation thrombolytic agents such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials. In Present scenario, it is observed that the heart diseases are increasing to a great extent, In India many youth celebrity are also loosing their life due to heart attack. Continued investigation in this area will provide new insights and promote progress towards the development of the ideal thrombolytic agent which are characterized by maximal coronary arterial thrombolysis with minimal bleeding.[4] The side effects are always there with synthetic drugs leading to therapeutic problem after long term use. Almost all the available thrombolytic agents are having their own significant shortcomings. Therefore, thrombolytic agents from natural sources like plants are considered to be less toxic and free from side effects than the synthetic ones.[5] Hence, the present research focus is to find out the safe, less or no side effect herbal agents with thrombolytic activity.[6] The present study has been carried out on hydro alcoholic extracts of the following two plant parts namely bulbs of *Allium cepa* and seeds of *Linum Usitatissimum.* Each plant extract is studied for its thrombolytic activity separately. Different extracts are taken together to formulate a poly herbal formulation, after optimization the polyherbal

* *Corresponding author.*
E-mail address: amiyaprusty@gmail.com (A. Kumar Prusty).

https://doi.org/10.18231/j.ijpp.2022.010

formulation is evaluated for its thrombolytic activity.

2. Material and Methods

2.1. Plant material

The plant parts of selected plants were collected from the local area near Salipur, Odisha. After proper authentication by a botanist, the voucher specimen was prepared and preserved. The collected plant parts were thoroughly cleaned, properly dried and powedered.

2.2. Preparation of hydro alcoholic extract

The powdered parts of the plant materials (100 gm) were extracted by maceration method with 1liter of hydroalcoholic solvent containing 70% ethanol for 72hrs. The procedure repeated three times at room temperature. The extract was concentrated at 50°C and was stored in the refrigerator till used for the study.[7] The yield of the extraction process was 36.7% (w/w).

2.3. Qualitative phytochemical screening of the extracts

The different plant extracts were subjected to qualitative chemical tests for detecting presence of different phytoconstituents lik earbohydrates, gums and mucilage, Proteins and amino acids, fixed oils and fats, alkaloids, glycosides, Flavonoids's, tannins and phenolic compounds, saponins etc.[8]

2.4. Preparation of sample dilution

Five different test solutions were used to evaluate the thrombolytic activity of the plant extract. The plant extract was dissolved in ethanol and shaken vigorously to prepare different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 μl of the ethanolic preparations of the plant were added to the micro-centrifuge tube containing the clots to check thrombolytic activity.[9]

2.5. Streptokinase (SK)

Commercially available lyophilized stac (Streptokinase) vial (Incepta Pharmaceutical Ltd) 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 study as standard.[7]

2.6. Thrombolytic activity

In vitro clot lysis activity of different extract was carried out according to following method. 5ml of venous blood was drawn from healthy volunteers (n = 3) and transferred to different pre weighed sterilized micro-centrifuge tube (1 ml/tube). The microcentrifuged tubes were incubated at 37°C for 45minutes to form clot. Serum was completely removed from the tubes without disturbing the clot formed and discarded. Each of the tube having clot was again weighed to determine the weight of the clot. The clot weight = weight of clot containing tube — weight of tube alone.

Each micro-centrifuge tube containing clot was appropriately labeled and 100 μl of the plant extract of different conentration was added to the tubes accordingly. As a positive control, 100 μl of streptokinase and as a negative non thrombolytic control, 100 μl of hydroalcoholic solution were added to the specific marked control tubes. The tubes containing clot and plant extract were incubated at 37°C for 90 minutes for observation of clot lysis. The experimental tubes were then centrifuged and the supernatant fluid was completely removed and discarded. The experimental tubes were again weighed to observe the difference in weight after clot disruption.[9] Finally, difference obtained in weight of the experimental tubes was calculated and the result was expressed as percentage of clot lysis by the following equation,

$$Percentage\ of\ clot\ lysis = \frac{Weight\ of\ released\ clot}{Clot\ weight} \times 100$$

2.7. Method of preparation of simple syrup (USP)

66.7 gm of Sucrose was weighed and added to purified water and heated until sugar dissolved completely with occasional stirring. Other additives like preservatives, colouring agent, flavouring agents etc were added as per the formula shown in following table. Decoction of plant extract added to the prepared simple syrup in a ratio of 1:5. Sufficient purified boiling water was added to the above mixture to a final volume of 100 ml.[10]

Table 1: Formulation of simple oral syrup 100ml

Name of the ingredients	Amount
Methyl parabeen sodium	0.2 g
Propyl parabeen sodium	0.1 g
Sodium benzoate	0.2 g
Sodium chloride	0.1g
Sugar	66.7g
Mixed fruit flavor	0.2ml
Colouring agent	0.2ml

2.8. Method of preparation of final herbal syrup

The decoction was prepared by taking equal proportion of the two extracts in a concentration of 10mg/ml each, mixed properly and filtered. The Filtrate obtained after filtration was taken for prepartion of the final herbal syrup. One part of sample mixture (decoction) was mixed with five parts

of prepared simple syrup i.e. in a ratio of 1:5. Required quantity of methyl paraben sodium, propyl paraben sodium, sodium chloride, sodium benzoate, were added and mixed properly, followed by addition of colouring and flavouring agents and mixed properly. Solubility was checked by observing the clarity of solution visually.[11] The final herbal syrup was then subjected to evaluation of production quality as per official standards, The In-vitro thrombolytic activity study was then carried out by the process stated before.

2.9. Evaluation of prepared herbal syrup

2.9.1. Physicochemical parameters

The prepared herbal syrup was evaluated for various physicochemical parameters such as physical appearance, pH, viscocity and Specific Gravity as per the guidelines of IP.

1. *Color examination:* Five ml of final syrup was taken in a clean watch glass and placed against white back ground under white tube light. The colour of syrup was observed by naked eye.
2. *Odor examination:* Two ml of prepared syrup was taken in a clean and dry test tube and smelled. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.
3. *Taste examination:* A little of prepared syrup was taken and its taste was filled by tongue.
4. *Determination of pH:* About 10ml of the prepared syrup was taken in a 100 ml volumetric flask and made up the volume up to 100 ml using distilled water. The solution was sonicated for 10 minutes. pH of the sonicated sample of syrup was measured with the help of digital pH meter.
5. *Specific gravity at 25^0C:* The Pycnometer was cleaned and dried properly and calibrated by filling it with recently boiled and cooled water at 25^0C and weighed. Assuming that the weight of 1 ml of water at 25^0C in air of density 0.0012 g/ml is 0.99602gm. The capacity of the Pycnometer was calculated. Adjusting the temperature of the prepared syrup to about 20^0C and the Pycnometer was filled with it. Then the temperature of the filled Pycnometer was adjusted to 25^0C, any excess syrup was removed and weighed. The tare weight of the Pycnometer was subtracted from the filled weight. The weight per milliliter was determined by dividing the weight in air, expressed in gm of the quantity of syrup which fills the Pycnometer at the specified temperature, by the capacity expressed in ml, of the Pycnometer at the same temperature. Specific gravity of the prepared syrup was obtained by dividing the weight of the syrup contained in the Pycnometer by the weight of water contained, both determined at 25^0C.[12]
6. *Determination of viscosity:* Brookfield viscometer was used to determine the viscosity of the prepared polyherbal syrup. The method was followed as per the standard operating procedure of the laboratory.

2.10. Stability testing

Stability testing of the prepared polyherbal syrup was performed on keeping the samples at accelerated temperature conditions. The prepared syrup was taken in amber colored glass bottles and was kept at accelerated temperature at 4^0C, Room temperature and 47^0C respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24 hr, 48 hr and 72 hr and observation for any change in the physicochemical parameters was determined.

3. Results and Discussions

3.1. Phytochemical screening

The phytochemical screening results were tabulated in Table 2. From the result it had been concluded that all the selected plants were having flavonoids, phytosterols, Tannins and phenolic compounds in common. Therefore, it could be predicted that the thrombolytic property might be due to presence of these phytochemicals.

Table 2: Results of phytochemical screening of selected plant extracts

Phytoconstituents	Allium C.Extracts	Linum U. Extracts
1.Carbohydrates	+	+
2.Gums & mucilage	-	+
3. Proteins & amino acids	-	+
4. Oils & fats	-	+
5. Alkaloids	+	-
6. Glycosides	+	+
7. Flavonoids	+	+
8. Tannins & phenolic	+	+
9. Saponins	+	-
10.Phytosterols & triterpenoids	+	+

(+ sign represent presence and –sign represent absence of particular phytoconstituent)

3.2. In-vitro thrombolytic activity

When the clots were treated with 100 μl each of different concentrations (2, 4, 6, 8 & 10 mg/ml respectively) of the test sample significant clotlysis activity was observed. The mean In-vitro thrombolytic activity and the results are presented in Table 3. Addition of 100μl Streptokinase, a positive control (30,000 IU), to the clots showed 64.97

± 0.82 percentage lysis of clot. On the other hand, hydroalcoholic solution was treated as negative control which exhibited a negligible percentage of clot lysis i.e.1.26 ± 0.23 98%.

The evaluation of the positive control (streptokinase) with negative control clearly demonstrated that clot dissolution does not occur when solvent was added to the clot. Encouraged by the result of the positive control, we compared five different concentrations of the test sample in the same manner with the negative control and observed significant thrombolytic activity. The in-Vitro thrombolytic activity study had shown that all the selected plants were having various degree of thrombolytic activity. *Allium C.* extracts shown higher activity followed by *Linum U.* extracts. Since phytochemical analysis showed that the crude extract contains tannin, flavonoids and phytosterols as common it could be predicted that these phytochemicals may be responsible for its clotlysis activity.

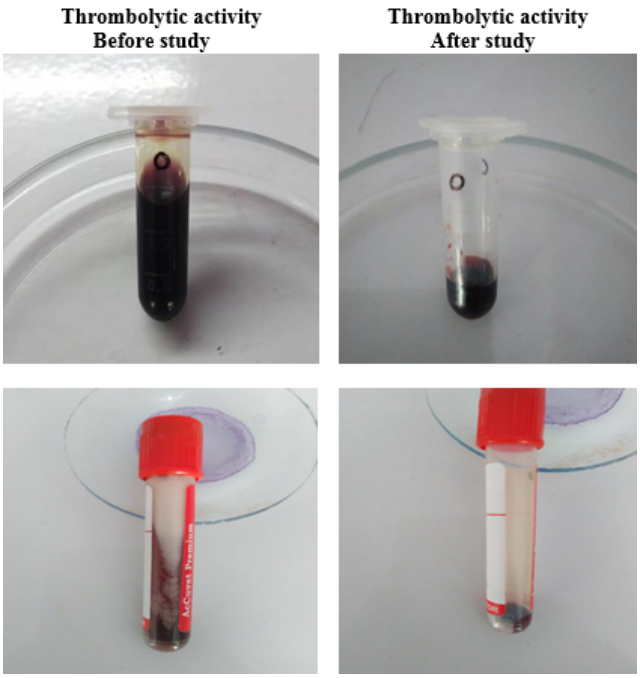

Fig. 1: Thrombolytic activity study of some treated samples

Table 3: Mean percentage clotlysis of different concentrations of selected plant extracts

Sample Extracts	2 mg/ml	4 mg/ml	6 mg/ml	8 mg/ml	10 mg/ml
Allium C.	10.61± 0.45	16.12± 0.99	24.28± 0.52	31.42± 0.78	38.93± 0.66
Linum U.	5.45± 0.68	8.68± 0.39	13.63± 1.15	17.89± 0.68	22.49± 0.93

3.3. Physicochemical parameters of prepared syrup

The prepared syrup was evaluated immediately after preparation and all the tested parameter along with turbidity/homogeneity were compared with the changes in accelerated stability testing. The final syrup found to have pH 7.05 and specific gravity 1.22 g/ml.

Table 4: Result of physicochemical parameters of developed poly herbal syrup.

Sl. No.	Physicochemical parameters	Observed Values
1	Color	Brownish red
2	Odor	Pleasant fruit odor
3	Taste	Sweet
4	pH	7.05
5	Wt/ml at 250C	1.165 g
6	Specific gravity	1.22 g/ml
7	Viscosity	0.11 ± 0.04 poise

3.4. In-vitro thrombolytic activity of prepared polyherbal syrup

The In-Vitro thrombolytic activity of polyherbal syrup was found to be more than individual extracts and can be comparable with that of positive control, Streptokinase as shown inTable 5.

Table 5: Percentage thrombolytic activity of polyherbal syrup

Sl No.	% In-Vitro thrombolytic activity
1	47.43
2	48.34
3	47.27
Mean± SD	47.68 ± 0.58

3.5. Stability study of prepared polyherbal syrup

The results of stability study of the polyherbal syrup had not shown any changes in the different tested physicochemical parameter as well as turbidity/homogeneity during 24 hr, 48 hr and 72 hr of observations. Thus, it can be concluded that the prepared poly herbal syrup may be used as a stable liquid dosage form and the results of the accelerated stability study may help for further studies of shelf-life of poly herbal syrup in near future.

4. Summary and Conclusion

Thrombosis is a critical event in the arterial diseases associated with myocardial infarction, anoxia, hypertension, stroke and venous thromboembolic disorders that account for considerable number of deaths worldwide in India streptokinase and urokinase are widely used due to lower cost as compared to other thrombolytic drugs. However,

these drugs have certain limitations such as hyperrisk of hemorrhage, severe anaphylactic reaction and lack of specificity, which cause serious and sometimes fatal consequences. Due to the shortcomings of availability of cheap and effective thrombolytic drugs, considerable efforts have been directed towards the discovery and development of thrombolytic substances from natural sources. Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are regarded as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development.

The plant extracts exhibited maximum clotlysis at a concentration of 10mg/ml. The comparison of the thrombolytic activity of the two different plants indicated that *Allium Cepa* exhibited a higher percentage of clot lysis. Analysing the phytochemicals in medicinal plants provides an insight to know how plants are medicinally effective and understanding the chemical composition leads to development of new medicines. The qualitative phytochemical screening of hydroalcoholic extracts shown presence of phytochemicals namely carbohydrates, amino acids, proteins, phenols, glycosides, saponins, quinones, flavonoids, tannins, volatile oils, terpenoids and alkaloids. The phytochemicals common in both the plant extracts may be responsible for the trombolytic activity. The prepared formulation might have important implications in cardiovascular health in developing countries where cheap and effective medicines are need of the hour. This investigation can also help in developing cost effective novel thrombolytic agents from common medicinal plant sources.

5. Source of Funding

None.

6. Conflict of Interest

None.

References

1. Sherwani SK, Bashir A, Haider SS, Shah MA, Kazmi SU. Thrombolytic Potential of Aqueous and Methanolic Crude Extracts of Camellia sinensis (Green Tea): In vitro study. *J Pharmacogn Phytochem*. 2013;2(1):125–9.
2. Fathima SN, Ahmad SV, Kumar BR. Evaluation of In Vitro Thrombolytic Activity of Ethanolic Extract of Curcuma Caesia Rhizomes. *Int J Pharma Res Rev*. 2015;4(11):50–4.
3. Sayeed MA, Kabir H, Rashid M, Bhuiyan M, Ahamad FM, Rashid MA. Thrombolytic Activity of Methanolic Extracts of Desmodium paniculatum (L.) and Sarcochlamys pulcherrima (Roxb. *Bangladesh Pharm J*. 2014;17(1):67–9.
4. Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator. *Ann Intern Med*. 1990;112(7):529–38. doi:10.7326/0003-4819-112-7-529.
5. Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue plasminogen activator. *J Am Coll Cardiol*. 1992;20(1):228–35. doi:10.1016/0735-1097(92)90164-i.
6. Wagnor H, Hikino H, Farnsworth R, London NR. The economic significance of plants and their constituents as drug. *Econ Med Plant Res*. 1989;3:1–17.
7. Masoud S, Leila S, Mohammadreza AG, Mojtaba R. Evaluation of fibrinolytic and antioxidant effects of Allium affine hydroalcoholic extract. *Res Pharm Sci*. 2017;12(4):299–306. doi:10.4103/1735-5362.212047.
8. Varinder S, Pawan K, Richa S. Extraction of antioxidant phytoconstituents from onion Waste. *J Pharmacogn Phytochem*. 2017;6(1):502–5.
9. Hussain MDS, Amin MT, Millat MS. In vitro thrombolytic potentials of methanolic extract of Vigna unguiculata Linn (seed). *J Pharmacogn Phytochem*. 2016;5(3):129–31.
10. Patel DA, Patel YK, Shah PB. Development and evaluation of herbal syrup from Neolamarckia cadamba Bosser leaves. *Int Res J Pharm*. 2012;3(9):201–2.
11. Shaheen S, Salma A, Amena A. Development and evaluation of herbal syrup from extract of fenugreek seeds. *J Pharm Res Ther*. 2020;10(2):111–4. doi:10.17605/OSF.IO/J4FG3.
12. Venkateswarlu K. In vitro Stability Testing of Syrup Dosage Form for Hepatitis. *Am J of Phytomedi Clin Ther*. 2013;6(1):491–7.

Author biography

Avinash Muduli, Student

Susanta Kumar Rout, Project Scientific-I

Amiya Kumar Prusty, Assistant Professor

Cite this article: Muduli A, Kumar Rout S, Kumar Prusty A. *In-vitro* thrombolytic activity study of a polyherbal formulation developed by using extracts of different medicinal plants. *Indian J Pharm Pharmacol* 2022;9(1):53-57.