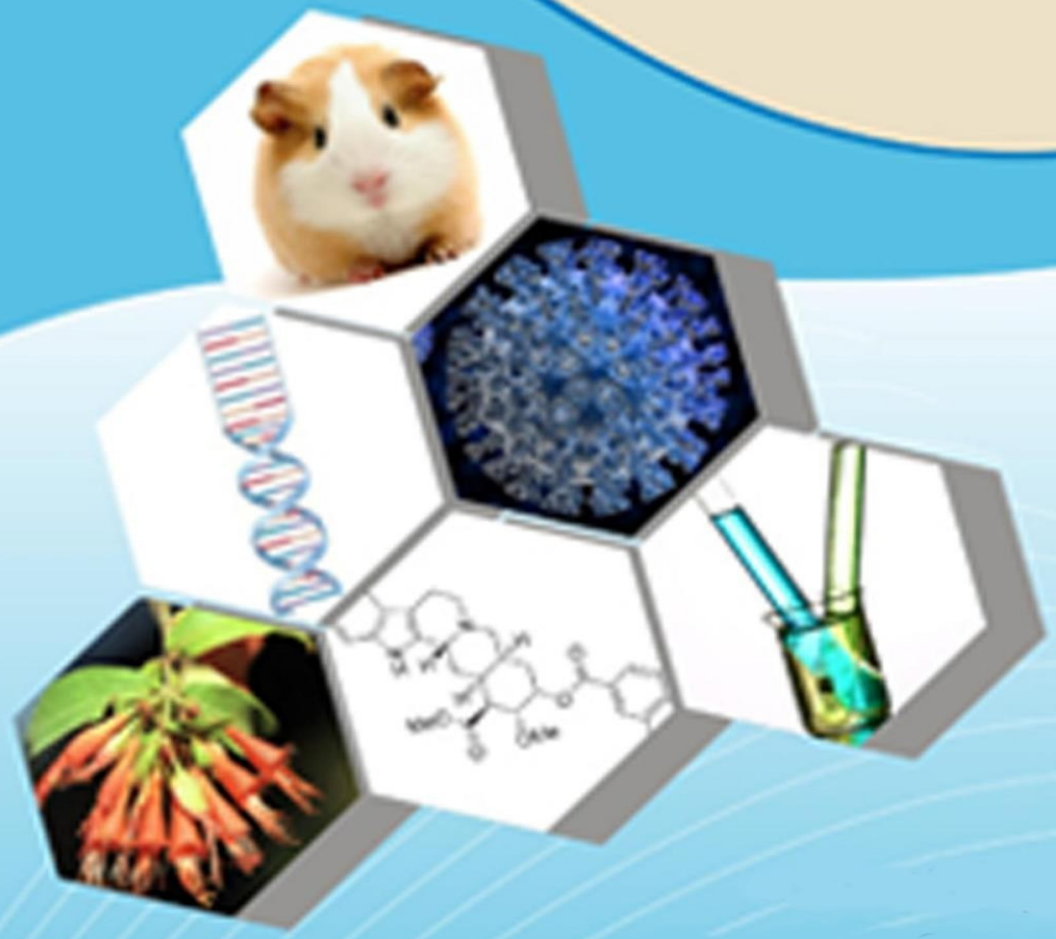
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In Vitro Antioxidant Activity of Polyherbal Syrup Prepared by Traditional Methods

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Abstract:

The goal of the current study was to assess the in-vitro thrombolytic efficacy of a polyherbal formulation made from extracts of several thrombolytically active medicinal plant parts, such as Linum Usitatissimum seeds and Allium cepa bulbs. Significant clot lysis activity was shown by the extracts at 38.93% and 35.65%, respectively, in relation to Streptokinase (64.97%) and water (1.26%). Good thrombolytic action was also shown by the polyherbal formulation made with these two extracts. Individual plant extracts' phytochemical analyses revealed the existence of many phytochemicals in common. This suggests that these phytochemicals may be the cause of the plants' in-vitro thrombolytic action.
Keywords: action of thrombolysis Plant extracts that are hydroalcoholic Polyherbal syrup Streptokinase The Thrombus

1. Introduction

A thrombus (blood clot) that forms in the circulatory system causes vascular occlusion, which may have fatal implications and result in thrombus-related disorders including acute myocardial or cerebral infarction. 1 Thrombolysis is the process of breaking up blood clots with therapeutic thrombolytic drugs. 2 Common thrombolytic medicines that break up clots include streptokinase, urokinase, and tissue plasminogen activator (tPA) such as alteplase and anistreplase. 3. Aspirin and heparin have a fairly effective rate of lysis acceleration and reocclusion prevention. Although death rates have been comparable to those of the few medications that have been the subject of extensive studies, patients with acute myocardial infarction benefit from the increased angiographic potency of selective third-generation thrombolytic medicines, such as monoteplase, tenecteplase, reteplase, etc. It is noted that in the current situation.

Heart disease is on the rise, and many young celebrities in India are losing their lives to heart attacks. Further research in this field will provide fresh perspectives and advance the creation of the optimal thrombolytic agent, which is defined by maximum coronary artery thrombolysis with the least amount of bleeding. 4. Synthetic medications usually have adverse effects, which might cause therapeutic issues with prolonged administration. Nearly every thrombolytic agent on the market has serious drawbacks of its own. As a result, thrombolytic medicines derived from natural sources, such as plants, are thought to be less harmful and to have no negative side effects than synthetic ones. 5. Therefore, the goal of current research is to identify herbal medicines with thrombolytic action that are safe, have few or no negative effects. 6. Hydro-alcoholic extracts of the following two plant parts—Allium cepa bulbs and Linum Usitatissimum seeds—were used in the current investigation. The thrombolytic activity of each plant extract is investigated independently. Following optimization, the thrombolytic activity of the polyherbal formulation is assessed. Various extracts are combined to create the formulation.

2. Material and Methods

2.1. Plant matter
Parts of a few chosen plants were gathered from the neighborhood around Salipur, Odisha. Following a botanist's correct verification, the voucher specimen was made and stored. The plant pieces that were gathered were carefully cleaned, dried, and powdered.

2.2. Hydroalcoholic extract preparation
One liter of hydroalcoholic solvent containing 70% ethanol was used for seventy-two hours to macerate the 100 grams of powdered plant material. At room temperature, the process was carried out three times. The extract was kept in the refrigerator until it was needed for the research after being concentrated at 50°C. 7. The extraction technique produced a 36.7% (w/w) yield.

2.3. The extracts' qualitative phytochemical screening
To identify the presence of various phytoconstituents, such as carbohydrates, gums, mucilage, proteins and amino acids, fixed oils and fats, alkaloids, glycosides, flavonoids, tannins and phenolic compounds, saponins, etc., the various plant extracts were put through qualitative chemical testing.

2.4. Sample dilution preparation
The plant extract's thrombolytic activity was assessed using five distinct test solutions. To create the test sample at various concentrations (2, 4, 6, 8, and 10 mg/ml, respectively), the plant extract was dissolved in ethanol and forcefully shaken. After being left overnight, the solution was decanted to extract the soluble supernatant, which was then filtered using a 0.22 micron syringe filter. To test for thrombolytic action, 100 µl of the plant's ethanolic preparations were introduced to the microcentrifuge tube that held the clots. 9.

2.5. Lyophilized stac (Streptokinase) that is commercially accessible

was extracted from three healthy volunteers and put into separate, pre-weighed, sterile microcentrifuge tubes (1 ml/tube). To create a clot, the microcentrifuged tubes 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]

2.1. Thrombolytic activity

In vitro clot lysis activity of different extract was carried out according to following method. 5ml of venous blood were incubated for 45 minutes at 37°C. Without affecting the clot that had formed, the serum was extracted from the tubes entirely and disposed of. To ascertain the weight of the clot, each tube containing the clot was weighed once again. The weight of the tube containing the clot divided by the weight of the tube alone is the clot weight. After properly labeling each microcentrifuge tube holding the clot, 100 µl of the plant extract at a varying concentration was applied to each tube. To the designated control tubes, 100 µl of streptokinase was added as a positive control and 100 µl of hydroalcoholic solution was added as a negative non-thrombolytic control. To observe clot lysis, the tubes containing the clot and plant extract were incubated for 90 minutes at 37°C. After centrifuging the experimental tubes, the supernatant fluid was extracted and disposed of. To determine the weight difference after clot breakup, the experimental tubes were weighed once again. 9. Ultimately, the weight difference between the experimental tubes was computed, and the outcome was represented as the percentage of clot lysis using the following formula:

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

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]

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

Table 1: Formulation of simple oral syrup 100ml

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^{0}C:* The Pycnometer was cleaned and dried properly and calibrated by filling it with recently boiled and cooled water at 25^{0}C and weighed. Assuming that the weight of 1 ml of water at 25^{0}C 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^{0}C and the Pycnometer was filled with it. Then the temperature of the filled Pycnometer was adjusted to 25^{0}C, 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^{0}C. [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^{0}C, Room temperature and 47^{0}C 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.

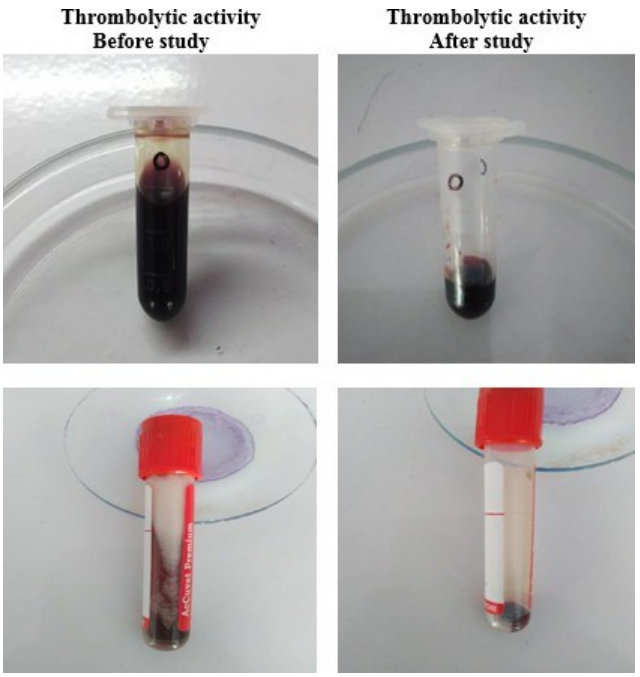


Fig. 1: Thrombolytic activity study of some treated samples

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.

At a concentration of 10 mg/ml, the plant extracts showed the highest level of clotlysis. Allium Cepa showed a greater percentage of clot lysis when the thrombolytic activity of the two distinct plants was compared. Understanding the chemical makeup of medicinal plants helps create novel medications, and analyzing their phytochemicals gives insight into how plants work as medicines. The presence of phytochemicals, including carbohydrates, amino acids, proteins, phenols, glycosides, saponins, quinones, flavonoids, tannins, volatile oils, terpenoids, and alkaloids, was shown by the qualitative phytochemical screening of hydroalcoholic extracts. The trombolytic action may be caused by the phytochemicals that are present in both plant extracts. In underdeveloped nations where affordable and efficient medications are desperately needed, the produced formulation may have significant effects on cardiovascular health. This research may also aid in the development of new thrombolytic medicines that are affordable and derived from popular medicinal plants.

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