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ANALYSIS OF *IN-VITRO* FIBRINOLYTIC ACTIVITY USING HERBAL EXTRACTS OF THREE AYURVEDIC PLANTS: *ALOE BARBADENSIS, HIBISCUS ROSA-SINENSIS* AND *CURCUMA LONGA*

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ABSTRACT

The aim of this experiment was to analyze the in-vitro fibrinolytic activity of Ayurvedic herbs, *Aloe Barbadensis* M., *Hibiscus rosa-sinensis* L. and *Curcuma longa* L. by using their herbal aqueous extracts on a dose dependent manner at different time intervals. This is to compare the efficacy of the herbal variants against the synthetic drugs used for treating thromboembolic disorders. As long-term medication of these, lead to lethal side effects of the body. Herbal extracts of Aloe vera, Hibiscus and *Curcuma longa* at time intervals of 24 hrs, 48 hrs and 72 hrs at 37℃ were observed. Streptokinsae (S.K) was chosen as a positive control and distilled water as a negative control. Percentage of clot lysis in 24 hrs for Aloe, Hibiscus and Curcumin were 38.60%, 26.93% and 22.34% respectively. In 48 hrs the readings were, 53.55%, 46.54% and 39.28% respectively. In 72 hrs it was, 73.24%, 62.56% and 52.96% respectively. Based on the results it was observed that the maximum clot lysis was noted with Aloe extracts at 72 hrs interval with 73.24% while the least lysis was observed in Curcumin with 52.96% at 72 hrs. Hence, it can be inferred that Ayurvedic extracts showed significant clot lysis with little to no side-effects possible and the relatively high lysis capacity with Aloe extract alone proves to serve as a better alternative to synthetic drugs in future.

Keywords: Fibrinolysis, Streptokinase, Haematology, Physiology, Phytopharmacology and Ayurveda

INTRODUCTION

Our body's nutrition, oxygen carrying capacity and waste removal is provided by the blood, which in humans is a permanently circulating fluid due to the numerous cells and proteins suspended in it. It's thicker than pure water. A healthy individual has about 5 litres of blood. The red coloured pigment of blood comes from the protein haemoglobin. A straw-coloured liquid called plasma makes up about half of the content of blood. Half of the blood volume is composed of cells like RBC's, WBC's and Platelets. Blood is conducted through blood vessels and is prevented from clotting in the blood vessels by the finely tuned balance of clotting factors. A blood clot is a clump of blood in a semi-solid state. Clotting is a necessary process that prevents one from losing too much blood from an injury or cut. A clot formed inside the veins, won't always dissolve on its own and can be a dangerous and life-threatening. An immobile blood clot is generally nonfatal but there's a chance that it could move and become dangerous. If a blood clot breaks free and travels through the veins up to the heart and lungs, it could get stuck and prevent blood flow, thereby causing a medical emergency.

There are different types of blood clots or fibrin clots and these can form in the blood vessels like veins or arteries. An arterial clot is a blood clot that occurs in an artery. This type of clot causes symptoms immediately and requires urgent treatment. Symptoms of an arterial clot include severe pain, paralysis of parts of the body, or both and can lead to a heart attack or stroke. A venous clot is a blood clot that occurs in a vein. These types of clots may build up more slowly over time but can still be life-threatening. Symptoms for a venous clot includes pain, swelling, redness and cramps to the limbs. The most serious type of venous clot is called a deep vein thrombosis (DVT). It is most likely to happen to one of the legs but could even happen to the arms, pelvis, lungs or even the brain. Risk factors of thrombos or blood clots are age (for people over 65 years of age), lengthy travels where people sit for a long time, obesity, pregnancy, family history of blood clots, smoking, cancer and certain birth control pills. Blood clots usually arise from a complex interaction of various mechanisms, including the activation of the coagulation, fibrinolytic systems, disruption of the vascular endothelium and the generalized activation of the cellular mechanisms resulting in clotting on the surface of monocytes and platelets in circulation **[1]**. Plasmin, plasminogen, plasminogen activator and fibrin are involved in the interaction as well **[2]**. The process of coagulation occurs via a capsule of sequential reactions requiring

several enzymes and other molecules known as coagulation (or clotting) factors.

Thrombolytic agents used are Anistreplase, Streptokinase, Alteplase, tPA, uPA and Warfarin. All thrombolytic agents have significant side effects and need large doses to be maximally effective, with limited fibrin specificity and no control over bleeding tendency, by free- flow of blood to avoid clots.

However, they are also implicated in the pathological progression of atherosclerotic lesions and arterial vascular thrombosis **[3]**. Uncontrolled platelet aggregation is critical in arterial thrombosis causing many disorders. Thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and myocardial infractions (heart attacks) which are the cause of morbidity and mortality in developed countries **[4]**. Thrombolytics are used to dissolve the fibrin of blood clots which are potentially life– threatening, especially those in arteries of the heart and lungs. It is also used against the clots formed in shunts during kidney dialysis and multiple pulmonary emboli. Therefore, thrombolytics or antithrombotic agents are considered as a key tool in the treatment and prevention of cardiovascular thrombotic diseases.

For activation of lysis and prevention of reocculusion, Aspirin and Heparin are significantly as effective as fibrinolytic drugs. The selective antiplatelet agents and thrombin inhibitor are most potent and drugs like Aspirin are most established as antithrombotic agents which still provides an effective secondary prevention of ischemic cardiovascular disorders. Yet safety is a main concern, as this drug can produce hemorrhagic events and upper gastrointestinal bleeding as major drawbacks **[5]**. Major investigative projects are being done in this field to discover more natural and effective therapeutics to dissolve clots. On the rising concern of health and safety, more people resort to naturally available drugs products. The context, concept and methods of the uses of natural products in the treatment of mankind have undergone remarkable changes **[6, 7]**. Due to the fact of such changes, natural medicines or traditional medicines made a revolutionary come-back with new strength and vigor to play a significant role in human health **[8, 9, 10]**. Use of herbs for treatment of disease has been in practice since ancient times. Herbal medicines are considered safer due to their natural activity. Extracts of natural products provide a useful source of bioactive compounds with advancement of phytochemistry and identification of new plants which can be used to develop as drugs directly or provide novel structure templates shows significant efficacy against diseases. Today a large proportion of drugs

in clinical use are produced by the synthesis of natural products and or their derivatives, while new mixtures are continually being discovered **[11, 12]**.

Ayurvedic medicine or "Ayurveda" comes from the Sanskrit terms Ayur (life) and Veda (knowledge) and is one of the world's oldest holistic healing systems. Ayurveda was practiced more than 5,000 years ago in India. The "University of Minnesota's Centre for Spirituality & Healing", claims it has gained popularity in the West as well. Drug formulation in Ayurveda is based on two principles; use as a single drug and use of more than one drug, in which the latter is known as "PHF". Polypharmacy or polyherbalism is known as a key traditional therapeutic herbal strategy that exploits the combining of several medicinal herbs to achieve extra therapeutic effectiveness. The revival of Ayurvedic PHFs have gained world attention due to its comparable efficacy, fewer side effects and better acceptability than allopathic drugs. Most of the time, they produce satisfactory effect and safety, making them one of the highly selected drugs of choice **[13]**. The present study involves a deep focus on herbal Ayurvedic extracts which may be used to dissolve clots. Three herbal Ayurvedic plants have been chosen; *Aloe barbadensis* M. (Aloe evra), *Hibiscusrosa-sinensis* L. (China rose) and *Curcuma longa* L. (Turmeric).

MATERIALS AND METHODS

Materials:

Blood Samples - The blood sample for the procedure was collected by venous puncture/phlebotomy. Whole blood (3ml) was drawn from healthy volunteers (n = 5) by a phlebotomist without a history of oral contraceptive or any other anticoagulant therapy. 500µl (0.5ml) of blood was transferred to each of the five previously weighed micro centrifuge tubes to form clots **[14]**. This was further used to analyze clot formation as well as clot lysis.

Aloe vera Leaves - Fresh leaves of Aloe vera were purchased from a local market selling medicinal plants. The leaves were collected in sufficient quantity (8 full length leaves) and cleaned well. They were identified to be the species *Aloe bardensis* M. and refrigerated until preparing the extracts.

Hibiscus Flowers - Fresh flowers were cleaned and identified to be the species *Hibiscus rosa-sinesis*. Then these were washed with distilled water and shade dried up to five days at room temperature.

Turmeric Rhizomes - Turmeric rhizomes were purchased from a local market selling traditional medicine. 50 grams was weighed and bought, they were identified to be the species *Curcuma longa* L.

Methods:

Preparation of Aloe vera extract - The gel was scraped off with a sterile spatula

and collected in a clean dish. This gel is then evaporated at 60ºC in a hot-air oven at six hours of interval for four days to get a solid dry mass. This was then converted into powder by grinding with a pestle and mortar. It was stored in a refrigerator for future use. Of which 1ml of this concentration was used for the procedure **[15, 16]**.

Preparation of Hibiscus extract - Fresh flowers which were plucked and were washed well in distilled water and air dried under shadow for five days. Ethanol extract was prepared by adding five grams of dried flowers and were kept in the 10ml ethanol solution. Then this mixture was ground with a mortar and pestle. The ground plant material was subjected to centrifugation for 10-15 minutes at 10,000rpm. The supernatant was collected and stored in a refrigerator for further studies, from which 1ml of this concentration was used in the alpine for the experiment **[17, 18]**.

Preparation of Curcumin extract - After cleaning, 50 grams of the rhizome was taken and air dried for 10 days, and then kept in a hot-air oven at 45°C at 72 hours at intervals of 7 hours. Then the dried rhizomes were ground. Glass extractor/Soxhlet apparatus was used for extraction process after the rhizomes were ground. 40gm of dried powder was left from 50gm of total product and this was taken in the glass extractor. 250ml of solvent methanol was added gradually & extraction was done. The suspension formed when 100 mg extract was suspended in 10 ml distilled water was shaken vigorously. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-micron filter paper. This was stored in a refrigerator. 1ml of this methanolic preparation was taken in each alpine tube. This method can be also called as the glass extraction or Soxhlet extraction of curcuminoids **[19, 20]**.

In vitro Thrombolytic Analysis - The thrombolytic activities of *Aloe barbadensis* M., *Hibiscusrosa-sinensis* and *Curcuma longa* L. were done by the method of in vitro clot lysis analysis technique, using streptokinase (S.K.) as a standard reference and distilled water as the negative control **[21]**.

Solution preparation - Commercially available lyophilized Stukinase (Streptokinase) vial of 15, 00,000 I.U., was procured from Smith Stocking & Co; Pharmacy, Chennai and then 5ml of sterile distilled water was added and mixed properly to form a homogenized solution. This suspension served as a stock and stored in a refrigerator, from which 100µl/0.1ml (30, 000 I.U) was used for in vitro thrombolysis **[22]**.

Bioassay; Procedure for clot lysis of the plant extracts - After preparation of the

plant extracts and S.K. solution, 0.5ml of each blood sample were distributed in five different tubes at a time, which was meant to observe lysis under different extracts, positive control (S.K) and negative control (distilled water) for each blood sample. These samples were left to incubate at 37ºC for 90 minutes for clot formation. After the clot formation had occurred, the serum was finely and completely aspirated without disturbing the clot and the tubes were weighed again to determine the clot weight **[23]**.

Formula applied–

Clot weight = Weight of the tube containing the clot – Weight of the empty tube.

Each Eppendorf tube containing the clot was labelled and 1ml of plant extracts were added to the tubes with the help of a micro pipette, after the first observation of 90 minutes, each of the three extracts (Aloe, Hibiscus and Turmeric) were added into the tubes, of 1ml concentration. This was to compare the maximum clot lytic activity among the different Ayurvedic herbs. As a positive control, 0.1ml of S.K was added to one of the labelled tubes and as a negative control, 0.1ml of distilled water was added separately to a previously labelled tube by using a 100µl micro pipette. All tubes were then incubated at 37ºC for three different time intervals/durations (24 hours, 48 hours and 72 hours).

Each day the clot lysis was recorded by carefully aspirating the serum formed from the lytic activity of the controls and the extracts and then weighing the current weights of the tubes in a mono-pan balance. This will eventually give us the clot disruption rate from the total clot weight of each individual sample, which was noted as percentage of the clot lysis.

Formula used –

Weight of released clot = Weight of clot before extract addition – Weight of clot after extract addition.

Clot weight = Weight of tube with clot – Weight of tube without clot

Percentage (%) of clot lysis = [Weight of released clot / Weight of clot] × 100

RESULTS

In the current study, the result findings suggest that, 0.1ml of S.K was used as a positive control had significant percentage of lysis **(Table 1)** with an average of over 90% clot lysis at 72 hrs **(Table 2)** at the end of the trial. In the first dose of 24 hrs, it showed 58.24% lysis. By the second interval of 48 hrs, 75.79% of lysis was noted and at 72 hrs of the last dose, 92.57% lysis was observed. While using 0.1ml of distilled water as negative control, results indicated barely enough clot dissolution. At 24 hrs with 08.96% lysis **(Table 1)**, at 48 hrs 23.33% lysis and at 72 hrs with 45.73% lysis.

In case of the herbal aqueous extracts, using 1ml concentration at an incubation of 24, 48 and 72 hrs for 5 different individual samples were as follows:

***Aloe Barbadensis* M.-** For 24 hrs of incubation the extract showed 38.60% lysis. At 48 hrs 53.55% and 72 hrs, 73.24% lysis was observed **(Table 1)**. Under statistical analysis, S.K and Aloe had a weak positive correlation of 0.041 for 24 hrs. a strong positive correlation of 0.769 for 72 hrs **(Table 3)**. The p value showed 0.00314 in 24 hrs which makes it highly significant and 0.00145 in 72 hrs **(Table 4)** also showing very high significance. Aloe and Hibiscus had a weak positive correlation of 0.070 for 24 hours while, it showed a weak negative correlation with -0.094 for 72 hrs **(Table 3)**. The p value showed a high significance of 0.01026 in 24 hrs and 0.00088 at 72 hrs **(Table 4)** making it very highly significant. Aloe and Curcumin had a weak negative correlation of -0.066 for 24 hrs and a strong negative correlation of -0.729 for 72 hrs **(Table 3)**. The p value observed as 0.00289 at 24 hrs, depicted a high significance and with 72 hrs showed 0.00106 **(Table 4)** also being highly significant.

***Hibiscus rosa-sinensis* L. –** For 24 hrs incubation, the extract showed about 26.93% lysis and at 48 and 72 hrs, 40.54% and 62.56% lysis were respectively noted **(Table 1)**. Under statistical analysis, S.K and Hibiscus extract showed a strong positive correlation of 0.679 at 24 hrs and positive correlation at 0.439 at 72 hrs **(Table 3)**. p value depicted a stark significance of 0.00492 at 24 hrs it was also highly significant at 72 hrs with 0.00757 **(Table 4)**. Hibiscus and Aloe extracts depicted a positive correlation at 24 hrs but a negative correlation at 72 hrs. While the p values at both incubations were highly significant. Hibiscus and Curcumin showed a strong negative correlation at 24 hrs and a positive correlation 72 hrs. p values at both times highly significant.

***Curcuma longa* L.-** Extracts at 24 hrs of incubation showed 22.34% lysis **(Table 1)** and at 48 and 72 hrs, 39.28% and 52.96% lysis were depicted respectively. According to statistical data, S.K and Curcumin extract showed a strong negative correlation with -0.711 at 24 hrs and repeated strong negative correlation with -0.741 at 72 hrs **(Table 3)**. The p values showed to be very highly significant at 24 hrs with 0.00083 and also still high at 72 hrs with 0.00037 **(Table 4)**. While Curcumin and Aloe had a weak negative correlation 24 hrs and a strong negative correlation for 72 hrs. Again, the p values proved to be highly significant at both times. Curcumin and Hibiscus extracts showed a very strong negative correlation at 24 hrs and a positive correlation at 72 hrs, with similar highly significant p values as the rest of the extracts.

Table 1: Percentage of clot lysis *in-vitro* - positive control, negative control, *Aloe barbadensis, Hibiscus rosa-sinensis* and *Curcuma longa* (under 24, 48 & 72 hours)

S. No:	Content	Incubation Time (hours)	Clot Lysis (%)
1.	Positive Control (S.K)	24 hours	58.24%
		48 hours	75.79%
		72 hours	92.57%
2.	Negative Control (Distilled water)	24 hours	08.96%
		48 hours	23.33%
		72 hours	45.73%
3.	*Aloe barbadensis*	24 hours	38.60%
		48 hours	53.55%
		72 hours	73.24%
4.	*Hibiscus rosa-sinensis*	24 hours	26.93%
		48 hours	40.54%
		72 hours	62.56%
5.	*Curcuma longa*	24 hours	22.34%
		48 hours	39.28%
		72 hours	52.96%

Table 2: Thrombolytic activity of different samples by time factor in terms of Mean ± Standard Deviation (n=5)

SAMPLES	MEAN ± S.D		
	24 hrs	48 hrs	72 hrs
S.K. (0.1ml)	58.24 ± 4.86	75.79± 6.14	92.57 ± 3.91
Aloe barbadensis (1ml)	30.60 ± 5.11	53.55 ± 5.14	73.24 ± 2.77
Hibiscus rosa-sinensis (1ml)	26.93 ± 2.92	40.54 ± 1.94	62.56 ± 3.01
Curcuma longa (1ml)	22.34 ± 3.40	39.28 ± 2.29	52.96 ± 4.62

Table 3: Correlation calculated with the different extracts and controls with respect to the highest and lowest incubation times

SAMPLES	CORRELATION	
	24 hrs	72 hrs
S.K &*Aloe barbadensis*	0.041	0.769
S.K &*Hibiscus rosa-sinensis*	0.679	0.439
S.K &*Curcuma longa*	-0.711	-0.741
Aloe barbadensis &*Hibiscus rosa-sinensis*	0.070	-0.094
Hibiscus rosa-sinensis &*Curcuma longa*	-0.992	0.162
Aloe barbadensis &*Curcuma longa*	-0.066	-0.729

Table 4: *t* – Test, two-tailed and paired values that were derived from the different extracts in different incubation times

	p – VALUES OBTAINED	
	24 hrs	72 hrs
S.K &*Aloe barbadensis*	0.00314**	0.00145**
S.K &*Hibiscus rosa-sinensis*	0.00492**	0.00757**
S.K &*Curcuma longa*	0.00083**	0.00037**
Aloe barbadensis &*Hibiscus rosa-sinensis*	0.01026*	0.00088**
Hibiscus rosa-sinensis &*Curcuma longa*	0.02714*	0.01352*
Aloe barbadensis &*Curcuma longa*	0.00289**	0.00106**

"*" Values are significant for the extracts & "" Values are highly significant for the extracts**

DISCUSSION

For treatment of diseases and (or) disorders, 30% of the pharmaceuticals are prepared from plant derivatives **[24]**. While there are plentiful thrombolytic drugs that can be obtained from various sources, some of which are modified further with use of recombinant DNA Technology **[25]**. But the side effects related to these drugs have been reported to have led to further

complications **[26]**. Some patients even die due to bleeding and embolism from heavy dosage of these synthetic enzymes **[27]**. Numerous research works were conducted to discover the plants that have antithrombotic effect and intake of them as medicine leads to prevention of coronary events and stroke **[28]**. Clot formation can be broken down by natural herbal extracts, thus reducing risk associated to consuming synthetic drugs **[29]**. In a most recent study, it was observed that Bougainvillea leaf extracts possessed an impressive lytic activity, with a similar analysis **[30]**.

Similarly, observations from the present study showed a satisfactory to significant clot lytic activity. Yet, the percentage of clot lysis was recorded to be maximum in case of maximum incubation time. As seen in the results, the performance of clot lysis was best with Aloe extract showing 73.24% lysis, which was the highest activity among the extracts in 72 hrs. Followed by Hibiscus extract with 62.56% lysis in 72 hrs. Curcumin extract showed comparatively low lytic activity to the other two extracts with 52.96% in 72 hrs. Aloe extract showed a strong positive correlation in 72 hrs with a 0.769 with S.K. While it had a weak negative correlation with both the Hibiscus extract and Curcumin extract in 72 hours. Depicting that they are inversely related in action (better lysis in one than other at a time). Aloe was analyzed to be highly significant with a p value of 0.00145 with S.K in 72 hours. It showed to be very highly significant with the other extracts as well. This shows that Aloe seems to be an active compound in clot dissolution and gave excellent results, with least error outcomes. While Hibiscus extract on the other hand also had a positive correlation with S.K **(Table 3)** and weakly negatively correlated with the extracts. It had a significant p value **(Table 4)** and was equally significant with the other extracts as well. Curcumin extract had a strong negative correlation with S.K. This was different from the other extracts and showed that the lytic activity was inversely related to the control at that time of interval. It had a weak negative and positive correlation with the extracts of Aloe and Hibiscus respectively. Through t-test, there was sufficient p values observed thereby proving that it too had least errors possible with very high significance **(Table 4)**.

Thus, through this investigation, it was proved that all the three Ayurvedic plants depicted significant clot lytic activity.

CONCLUSION

In conclusion from the recorded data, it can be demonstrated that the findings may have significant implications in cardiovascular health. In addition, the findings may indicate the possibility of developing new

and novel thrombolytic compounds from *Aloe barbadensis* M, *Hibiscus rosa-sinensis* and *Curcuma longa* L plant extracts. This will give rise a new era of drug docking with new target molecules and production of advanced therapy by the use of these natural extracts. In this *in-vitro* study, it can be demonstrated that Ayurvedic medicine could be as effective as modern medicine to reduce risk of cardiogenic problems. This proves the objective that clot lysis can be achieved by these Ayurvedic herbal preparations at a longer time factor on a dose-dependent manner. However, this is a preliminary study, yet to be investigated on clinical trials (*in-vivo*), isolation of enzyme activity and compounds responsible for fibrinolysis in order to invent new safe, cost-effective and easily acquirable drugs.

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REFRENCES

[1] Rickles F.R. and Falanga A. Pathophysiology of thrombophilic state in cancer patients.Semin. Thromb. Hemostat. 1999; 25(2): 173-82.

[2] Alkjaersig N., Fletcher A.P. and Sherry S. The mechanism of clot dissolution by plasmin.J. of Clin. Inves. 1959; 38, 1086.

[3] May A.E., Seizer P. and Gawaz M. Platelets: inflammatory firebugs of vascular walls. Aterioscl. Thromb. Vascular Biol. 2008; 28(3): 5-10.

[4] Dickneite G., Seiffe D., Diehl K.H., Rogers M. and Czech J. Pharmacological characterization on a new 4-amidinophenylalanine thrombin-inhibitor (CRC220). Thromb. Res. 1995; 77:357-368.

[5] Johnsen S.P., Sørensen H.T., Mellemkjoer L., Blot W.J., Neilsen G.L., McLaughlin J.K. and Olsen J.H. Hospitalization for upper gastrointestinal bleeding associated with use of oral anticoagulants. Thromb Haemost. 2001; 86(2): 563-568.

[6] Sumi H., Hamada H., Nakanishi K. and Hiratani H. Enhancement of fibrinolytic activity in plasma by oral administration of nattokinase. Acta Haematologica. 1990; 84(3):139- 143.

[7] Gesler W.M. Therapeutic landscapes: medical issues in light of the new cultural geography. Soc. Sci. Med. 1992; 34: 735–746.

[8] Demrow H.S., Slane P.R. and Folts J.D. Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine

coronary arteries. Circu. 91: 1182-1188.

[9] Briggs W.H., Folts J.D. and Osman H.E. Administration of raw onions inhibits platelet-mediated thrombosis in dogs. The J. of Nutri. 2001; 131: 2619-22.

[10] Yamamoto J., Yamada K., Naemura A., Yamashita T. and Arai R. Testing various herbs for antithrombotic effect. Nutri. 2005; 2: 580–587.

[11] Van Wyk B.E. and Smith G. Guide to the Aloes of South Africa. Briza Publications. 1996; 8-20.

[12] Rahman A., Choudhary M.I and Thomsen W.J. Bioassay techniques for drug development. Harward Acad. Pub. 2001; 1-3.

[13] Parasuraman S., Thing G.S. and Dhanaraj S.A. Polyherbal formulation: Concept of Ayurveda. Pharmacog. Rev. 2014; 8(16): 73–80.

[14] Kawsar M.H., Sikder M.A., Rana M.S., Nimmi I. and Rashid M.A. Studies of Thrombolytic and cytotoxic properties of two asteraceous plants of Bangladesh. Bangladesh Pharmaceut. J. 2011; 14: 103-110.

[15] Waller T.A., Pelley R.P. and Strickland F.M. Industrial processing and quality control of *Aloe barbadensis* (Aloe vera) gel, Aloes: The genus Aloe. Boca Raton Publ. 2004; Ch- 8:68.

[16] Subramanian K., Narmadha S., Vishnupriya U. and Vijayakumar V. Release characteristics of Aspirin and Paracetamol drugs from tablets with Aloe Vera gel powder as a drug carrier. Drug Inven. Today. 2010; 2(9): 424-428.

[17] Renuga G. Screening for their effect on Fibrinolytic properties. W. J. of Pharm. Pharmaceut. Sci. 2008; 10: 43-46.

[18] Aruna A., Mohamed Halith S., Jeya Sundari K. and Abirami A. Fibrinolytic Activity in Medicinal Plants. W. J. of Pharm. Pharmaceut. Sci. 2016; 6(1): 943-948.

[19] Irfan Newaz Khan, Razibul Habib Md., Mominur Rahman Md., Adnan Mannan, Mominul Islam Sarker Md. and Sourav Hawlader. Thrombolytic potential of *Ocimum sanctum* L., *Curcuma longa* L., *Azadirachta indica* L. and *Anacardium occidentale* L. J. of Basic Clin. Pharm. 2011; 2(3): 125-127.

[20] Pranali Waghmare, Dheeraj Patingrao, Pramod Kadu and Bhanuben Nanavati, Extraction, Isolation, Purification and Identification of Curcumin: A Review Article. Europ. J. of Biomed. Pharmaceut Sci. 2015; 2(3): 108-123.

[21] Prasad S., Kashyap R.S., Purohit H.J., Taori G.M. and Daginawala H.F. Effect of *Fagonia arabica* (Dhamsa)

on, *in-vitro* thrombolysis. BMC Compl. Alter. Med. 2007; 7(36): 1-7.

[22] Chowdary S.N., Badrul A.M., Haque A.S.M.T., Zahan R. and Mazumder M.E. In-vitro Free Radical Scavenging and Thrombolytic activities of Bangladeshi Aquatic Plant Aponogeton undulates Roxb. Golden J. of Pharm. 2011; 5(1): 27-32.

[23] Sarker R., Sharmin T., Chowdhury S.R. and Islam F. Thrombolytic Activity and Preliminary Cytotoxicity of Five Different Fractions of Methanol Extract of *Allamanda cathartica* Leaf. J. of App. Pharmaceut. Sci. 2012; 2(7): 129-132.

[24] Saikat Sen and Raja Chakraborty. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. J. of Trad. Compl. Med. 2017; 7(2): 234–244.

[25] Verstraete M. Third Generation thrombolytic drugs. Amercn. J. of Med. 2000; 109(1): 52-58.

[26] Baruah D.B., Dash R.N., Chaudari M.R. and Kadam S.S. Plasminogen activators: A comparison. Vascular Pharm. 2006; 44: 1-9.

[27] Gallus A.S. Thrombolytic therapy for venous thrombosis & pulmonary embolism. Bailliere's Clin. Haemat. 1998; 11(3): 663-673.

[28] Nor N.H.M., Othman F, Tohit E.R.M. and Sabariah Md. Noor. Medicinal Herbals with Antiplatelet Properties Benefit in Coronary Atherothrombotic Diseases. Thromb. 2016; 5952910: 1-7.

[29] Bhattacharya S., Victoria A. Ploplis and Francis J. Castellino. Bacterial Plasminogen Receptors Utilize Host Plasminogen System for Effective Invasion and Dissemination. J.of Biomed. Biotech. 2012; 81: 278–284.

[30] Aparna Suresh and Mubeen Sultana D. Evaluation of *In-Vitro* Thrombolytic Activity Using *Bougainvillea Spectabilis* Aqueous Leaf Extract Under Different Concentrations. Int. Res. J. of Pharm. 2019; 10(7):1-5.