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ORIGINAL ARTICLE

Evaluation of *In-vitro* anti-hemolytic and fibrinolytic activity of *Sansevieria cylindrica* and *Plumeria obtusa* plants using different methods

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ABSTRACT

Sansevieria cylindrica and Plumeria obtusa plants are traditionally used by the local healers during intentional and unintentional accidental injuries. However, few studies have been conducted to evaluate the pharmacological activities of these plants. The current study aimed at investigating the in-vitro anti-hemolytic and fibrinolytic activity of both plant extracts. The plants were collected and authentication was performed. Phytochemical screening was done thereafter. Sansevieria cylindrica leaves and Plumeria obtusa seeds extraction was performed using water and ethanol in combination. The anti-hemolytic activity was assessed using 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride, and Hydrogen peroxide induced hemolysis of RBCs. The fibrinolytic potential was evaluated by clot degradation and clot weight determination method. The dose-dependent increase in anti-hemolytic activities was reported by both plants. The percentage inhibition was higher in the case of the combination of plants mostly in a non-significant manner. The rate of clot lysis by Sansevieria cylindrica was high using the clot degradation method, whereas it was found slightly higher by Plumeria obtusa with clot weight determination. However, the difference was significant in both cases ($p < 0.001$) compared to standard at higher concentrations. The hydroalcoholic extracts of Sansevieria cylindrica and Plumeria obtusa individually and also as 1:1might be responsible for anti-hemolytic and fibrinolytic activities.

Keywords: Anti-hemolytic, Fibrinolytic, Hemolysis, Plumeria obtusa, Sansevieria cylindrica.

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INTRODUCTION

Hemolysis is an important indicator of free radical-induced membrane damage. Reactive oxygen species (ROS) attack on erythrocyte membrane, causes lipid peroxidation, oxidation of protein present in the membrane, and lysis of erythrocytes by changing hemoglobin structure [40, 19]. Such morphological changes and oxidative damage are usually observed in erythrocyte disorders and other diseases, including Alzheimer's disease, sickle cell anemia, renal failure, and β-thalassemia, as well as during cellular aging [1, 3, 38]. Although, there is a powerful defense system for the prevention of oxidation, erythrocytes of such patients demonstrate an increased ROS and decreased content of antioxidants than their normal counterparts [35]. Therefore, it is necessary to focus on the use of substances to inhibit the damage by free radicals.

Thrombus development inside the blood vessels inhibits the bloodstream through the circulatory system leading to high blood pressure, the stroke of the heart, anoxia, atherosclerosis, angina, ischemic heart disease, thromboembolism, myocardial and cerebral infarction [36]. Venous thromboembolism (VTE) consisting of serious and life-threatening complications such as deep vein thrombosis, post-thrombotic syndrome, and pulmonary embolism (PE), is of great clinical concern worldwide (Safaeian et al. 2017). In Western countries, the incidence rates of VTE and PE are approximately 0.87–1.82 per 1000 person-years and 0.45–0.95 per 1000

person-years, respectively (Nicholson et al. 2020). In the United States, VTE events are estimated to cost US healthcare more than 7 billion dollars each year [2, 9]. Despite different pharmacological treatments available including antithrombotic and fibrinolytic drugs, venous thromboembolism remains a major cause of mortality and disability. Therefore, investigations for finding efficacious and safe antithrombotic drugs with a low risk of bleeding are ongoing [23].

Complementary and alternative medicine is one of the emerging fields in health care today. The use of plants as medicine is ancient practice and Indians, and other communities are acquainted with it since 5000 B.C. [24]. Worldwide many plants in the form of basic plant extracts/formulations are utilized for their medicinal properties against various diseases [25]. Many plants contain compounds that have been evidenced to stabilize the RBC membrane by scavenging free radicals and might possess an anti-hemolytic effect on human erythrocytes [17]. Also, use of herbal medicine as one of the potential sources of prevention and treatment of venous thromboembolism has been reported [16].

Sansevieria cylindrica plant belongs to the Asparagaceae family and is commonly found in the African continents whereas it is also grown for ornate reasons in Egypt, India, and other countries [30-34, 2]. Sansevieria species exert a wide range of biologic activities like antitumor, antibacterial, scavenging free radicals, antidiabetic effects, and suppression of capillary permeability which might help in anti-inflammatory activity [5]. *Plumeria obtusa* plant is geographically distributed in Greater Antilles, Florida, northern Central America, and southern Mexico belonging to the Apocynaceae family [6, 7]. Plumeria has many medicinal uses such as emmenagogue, febrifuge, hemostatic, laxative, purgative, rubefacient, stimulant, and vermifuge [8, 9].

There are very few reports that demonstrate their biological activities. In our research, it has been observed that hydro-alcoholic extracts of these plants contained a good amount of phenolic compounds and flavonoids that might explain these anti-hemolytic and fibrinolytic activities. This study aims to assess the *in-vitro* anti-hemolytic activity by spectroscopy and fibrinolytic activities by clot degradation and clot weight method using hydro-alcoholic extracts of the plants.

MATERIAL AND METHODS

Collection and authentication of plant material

The fresh whole plants of *Sansevieria cylindrica* and *Plumeria obtusa* were collected from the Mehenduri village of Tehsil-Akole, District-Ahmednagar, Maharashtra, India. The leaves and seeds of plants respectively were then subjected to washing to clear dust matters and unwanted debris. The herbariums of the plants were prepared and were sent to the Western Regional Center, Pune, Maharashtra, India, which is affiliated with the Botanical Survey of India (BSI), the apex taxonomic research organization under the Government of India. The leaves and seeds were shade dried and crushed into powder using an electrical grinder, then sieved by using 20 mesh and stored in an air-tight container for long-term use.

Hydro-alcohol soluble extraction

The leaves and seed powder were weighed and dissolved in 100 ml of water and ethanol (water: ethanol 40:60). The drugs were shaken for 24 hours at 100 rpm on a rotary shaker. In a weighed porcelain dish, 25 ml of the filtrate was allowed to evaporate for drying after filtration. In the end weight difference was calculated and extractive values were expressed in % (w/w) [10].

Qualitative and quantitative phytochemical analysis

Qualitative phytochemical screening of the extracted plant material was performed as recommended by the standard method [11]. These tests were conducted to determine the presence or absence of carbohydrates, proteins, fats, fixed oils, flavonoids, glycosides, alkaloids, tannins, and saponins.

Assessment of total phenolic content

In the current study, the Folin-Ciocalteu reagent was used to determine the total phenolic content of plant extracts [20]. 2.5 ml Folin-Ciocalteu reagent (1:10 dilution) and 2 ml of Na_2CO_3 (7.5 % w/v) were added to 0.5 ml of plant extract of different concentrations (20, 40, 60, 80, 100, 120 µg/ ml) and incubated at room temperature for 15 min. The absorbance was measured at 750 nm using a UV-visible spectrophotometer. Gallic acid dissolved in methanol remained standard, and results were stated as µg of Gallic acid equivalent per mg of extract (µg GAE/ mg extract).

Assessment of total flavonoid content

The method reported earlier (Maswada 2013) was used to determine the total flavonoid content of plant extracts by colorimetry. Extracts and standard solutions of Quercetin (10, 20, 30, 40, 50, 60 µg/ml) were added to distilled water (4 ml) in a volumetric flask (10 ml). To the flask, after each 5min break, 5% $NaNO_2$ (0.3 ml), 10% $AlCl_3$ (0.3 ml), and 1M NaOH (2 ml) were added, respectively, and then a volume of 10 ml was made up with distilled water. The absorbance of this solution was measured at 510 nm against blank. The results were expressed as µg of Quercetin equivalent per mg of extract (µg QE/ mg extract).

Assessment of *In-vitro* anti-hemolytic activity

Preparation of erythrocytes suspension

5 ml of blood was collected from a healthy male rat in an EDTA tube. The blood was centrifuged at 1500 rpm for 3 min. Plasma (supernatant) was discarded and the pellets were washed three times with sterile phosphate buffer saline (PBS) solution (pH 7.2 ± 0.2). The cells were resuspended in normal saline (0.5%).

Anti-hemolytic activity using AAPH reagent

The *in-vitro* anti-hemolytic activity was performed using the method given by dos Santos et.al. 2018 with little modification (dos Santos et al. 2018). A volume of 0.5 ml of the cell suspension (prepared above) was mixed with 0.5 ml of the different concentration of hydro-alcoholic extracts (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of both the plants separately, solubilized in dimethyl sulfoxide (DMSO; final concentration up to 0.08%). After this period, 50 mM AAPH solution was added to the groups subjected to hemolysis induction. This mixture was incubated at 37°C for 4 h with frequent stirring. Erythrocytes incubated with PBS were used as the negative control. The reaction mixture except plant extract was used as the positive control. After every 60 min of incubation, the samples were centrifuged at 4000 rpm for 10 min, and an aliquot was collected from the supernatant, followed by dilution in PBS. The absorbance of samples was measured at 545 nm. The assay was performed in triplicate. The percentage inhibition of hemolysis was calculated using the formula;

% inhibition of hemolysis = (OD of test - OD of negative control / OD of positive control - OD of negative control) x 100

Anti-hemolytic activity using H2O2 reagent

The anti-hemolytic potential of extracts was executed by spectrophotometry as described by Afsar et.al. 2016 with minor changes [1]. 1 ml of the extracts of both the plants (0.2,0.4,0.6,0.8,1.0 mg/ml in PBS) were dispensed with 0.2 ml of erythrocyte suspension (prepared above) and incubated at room temperature for 20 min. To this, 0.1 ml of H2O2 solution made in buffered saline was added for provoking oxidative degradation of the membrane lipids. Subsequently, the samples were centrifuged at 1000 × g for 10 min and the absorbance of each supernatant was noted spectrophotometrically at 540 nm. The hemolysis using the H_2O_2 reagent was used as the positive control. For negative control erythrocytes suspended in phosphate buffer saline were used. Each set of experiments was performed in triplicate and the inhibitory activity of different extracts was calculated and expressed as percent inhibition of hemolysis by the above-given formula. Quercetin was used as a reference standard and a similar procedure was followed for the combination (1:1) of both plant extracts in both assays.

Assessment of *In-vitro* fibrinolytic activity

Fibrinolytic activity using clot degradation

The fibrinolytic activity of the plant extracts was observed by artificial blood clot degradation (Klafke et al. 2012). An artificial blood clot was made by spontaneous coagulation of 50 µL of fresh rat blood in a tube. One hour later, the artificial blood clot was rinsed out repeatedly. The artificial blood clot was dipped in 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml of plant extracts or Streptokinase (reference standard) at room temperature. Saline was used as a negative control. The degradation of the clot was estimated by color development after 1h at room temperature. Then red blood cells were lysed by adding 20 µL of triton 5% and then the absorbance of the final solution was read at 560 nm. The clot with triton was used as the positive control. The amount of color was estimated by linear regression analysis. The percentage fibrinolysis was calculated using the formula;

% fibrinolysis = (OD of test - OD of negative control / OD of positive control - OD of negative control) x 100

Fibrinolytic activity using clot weight

The fibrinolytic activity was quantitatively evaluated by the measurement of clot weight [30, 2]. Whole blood samples were collected. The blood specimen (400 µl) were moved to previously-weighed sterilized micro-centrifuge tubes and incubated at 37°C for 30 min to form clots. After the formation of the clot, serum was completely removed without disturbing the clot and each tube was again weighed to determine the weight of the clot. Different concentration of plant extracts (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) were added to micro-centrifuge tubes containing the clots. Streptokinase was used as a reference control and sterilized normal saline as a negative non-thrombolytic control. After incubation of all tubes at 37ºC for 90 minutes, the released fluid was removed from the tubes and again weighed and the percentage of clot lysis was determined. A similar procedure was followed for the combination (1:1) of both plant extracts in both methods. The % clot lysis was calculated by the formula;

% clot lysis = (Weight of final clot / Weight of initial clot) x 100

Statistical analysis

All data were analyzed statistically. Results are expressed as mean ± standard deviation. The difference among experimental sets was compared by two-way analysis of variance (ANOVA) followed by multiple comparisons. The differences were considered to be statistically significant when $p < 0.05$ and referred to as statistically highly significant when $p < 0.001$.

RESULTS

Authentication of plant and its extractive value

Fresh plants were collected and the BSI has authenticated the same based on the herbarium and the available database. It has provided the authentication certificate vide no. BSI/WRC100-1/TECH/2019/62 dated 19th Dec.2019 and confirmed that, the submitted plant species are *Sansevieria cylindrica* Bojer ex Hook. and *Plumeria obtusa* L. belonging to the family of Asparagaceae and Apocynaceae, respectively.

Extractive value

The hydro-alcoholic extractive values of *Sansevieria cylindrica* leaves and *Plumeria obtusa* seeds were found to be 9.14, and 10.16 (%w/w), respectively.

Phytochemical analysis

Preliminary phytochemical screening reported the presence of alkaloids, glycosides, steroids, flavonoids, and tannins, as shown in Table 1. The phenolic content in *Sansevieria cylindrica* extract was 84.2 µg GAE/mg of extract whereas, flavonoid content was found to be 56.9 µg QE/mg of extract. In the case of *Plumeria obtusa,* the phenolic content was 79.69 µg GAE/mg of extract, whereas flavonoid content was 37.97 µg QE/mg of extract.

Table 1: Qualitative phytochemical analysis of plants

S.N	Parameters	Method	Positive (+) / Negative (-)	
			Sansevieria cylindrica	*Plumeria obtusa*
1.	Carbohydrates	Molish test	+	+
		Fehling solution test	-	-
		Benedict's	+	+
2.	Amino acids	Ninhydrin test	-	-
3.	Proteins	Biuret	-	-
4.	Flavonoids	Shinoda test	+	+
		Zn. Hydrochloride test	-	-
		Lead acetate test	+	-
5.	Alkaloids	Dragendroff's test	+	+
		Mayer's test	+	+
		Hager test	-	+
		Wagner's test	+	+
6.	Glycosides	Borntrager's test	-	-
		Keller Killani test	+	+
7.	Volatile oil	Stain test	-	-
8.	Fixed oils and fats	Spot test	-	-
9.	Steroids	LibermannBuchard test	+	+
		Salkowski test	-	-
10.	Saponins	Foaming test	+	-
11.	Tannins and phenols	FeCl3 test	-	+
		Potassium dichromate test	+	+

Inhibition of Hemolysis

This experiment aimed to assess whether *Sansevieria cylindrica* and *Plumeria obtusa* plant extracts were able to prevent oxidative damage caused by different reagents to the erythrocyte membrane. Figure 1 shows the in-process anti-hemolytic activity using plant extracts.

Anti-hemolytic activity using AAPH reagent

The protective effect of plant extracts against hemolysis was assessed in this study using an AAPH reagent. The *Sansevieria cylindrica* and *Plumeria obtusa* hydro-alcoholic extract reported 72.88% and 68.02% inhibition of hemolysis, respectively at the highest concertation (1.0 mg/ml) used in the study. The percentage inhibition of hemolysis was found to be increased with the increasing concentration of plant extracts. However, the comparison of *Sansevieria cylindrica* and *Plumeria obtusa* plant extract with reference standard Quercetin reported a statistically significant difference ($p < 0.001$) at the concentration of 1.0 mg/ml. Both plants extract in combination also showed stabilization of erythrocytes with minimum damage due to oxidative stress and the analysis specified no statistically significant difference ($p > 0.05$) between the standard and combination. In contrast, this combination reported highly significant hemolysis as compared to the individual plant. Thus, it shows that the combined effect of extracts was larger than the additive effect of the individual plant. (Table 2) The percentage inhibition of hemolysis induced by AAPH using plant extracts and standard is displayed in Figure 2.

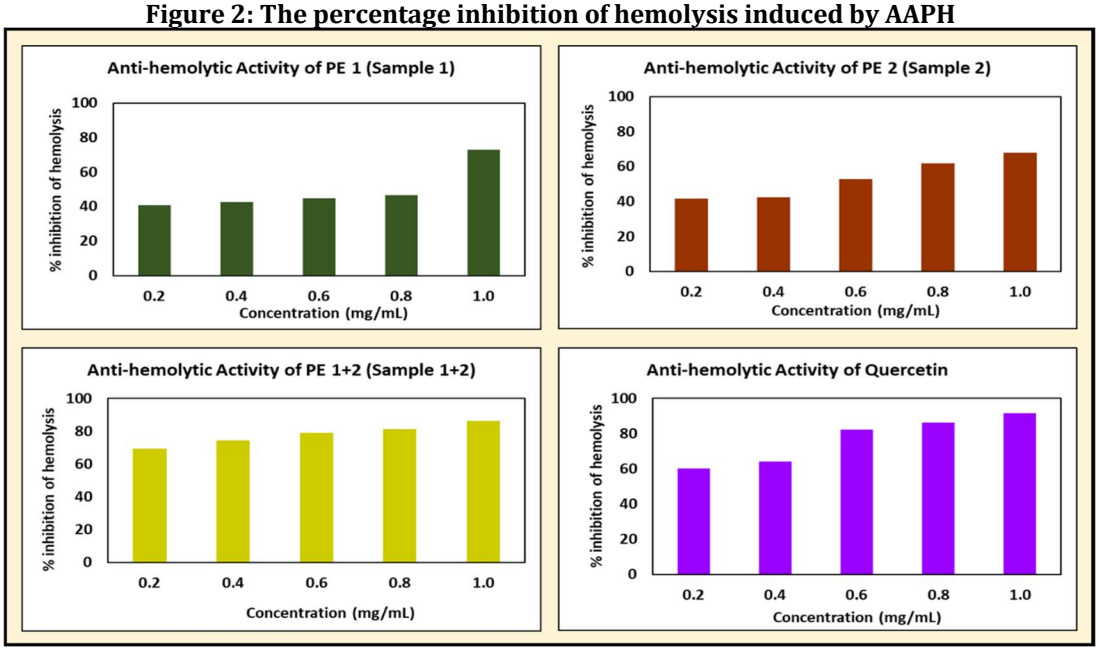
Figure 1: In-process anti-hemolytic activity using plants extracts; **a:** Assebling the chemicals, test tubes and other materials for initiating the study; **b:** Sample 1 with various concentration after addition of reagent; **c:** Sample 2 with various concentration after addition of reagent; **d:** Sample 1+2 with various concentration after addition of reagent.

Table 2: The observations for AAPH-induced hemolysis activity (n=3)

Conc. (mg/ml)	Sample 1		Sample 2		Sample 1+2		Standard	
	OD	% inhib.	OD	% inhib.	OD	% inhib.	OD	% inhib.
0.2	2.09 ± 0.01	40.92± 0.39	2.10 ± 0.01	41.53 ± 0.27	2.82 ± 0.05	69.55± 1.97	2.58 ± 0.09	60.11± 3.60
0.4	2.13 ± 0.01	42.47 ± 0.17	2.13 ± 0.01	42.51 ± 0.22	2.94 ± 0.08	74.44 ± 3.17	2.67 ± 0.07	63.93± 2.69
0.6	2.19 ± 0.01	44.79 ± 0.18	2.39 ± 0.02	52.67 ± 0.78	3.06 ± 0.01	78.93 ± 0.32	3.14 ± 0.05	82.23± 1.96
0.8	2.24 ± 0.01	46.75 ± 0.05	2.62 ± 0.05	61.74 ± 1.88	3.12 ± 0.01	81.27 ± 0.14	3.24 ± 0.02	86.17± 0.72
1.0	2.91 ± 0.03	72.88 ± 1.19	2.78 ± 0.08	68.02 ± 3.32	3.25 ± 0.04	86.30 ± 1.71	3.38 ± 0.04	91.66± 1.63

** Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seeds extract*

Figure 2: The percentage inhibition of hemolysis induced by AAPH



** PE: Plant extract; Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Anti-hemolytic activity using H_2O_2 reagent

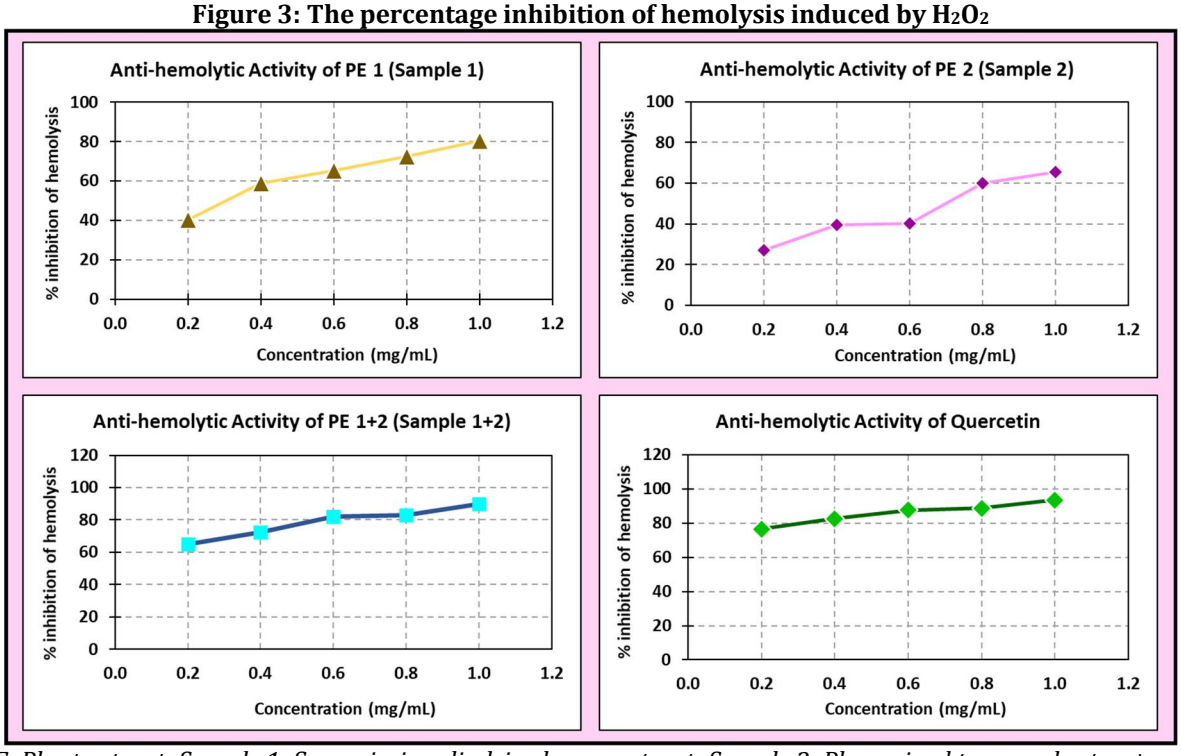
The anti-hemolytic activity of crude extracts of *Sansevieria cylindrica* and *Plumeria obtusa* was screened against damaged erythrocytes. Both the plant extracts exhibited a dose-dependent pattern of anti-hemolytic effect toward erythrocytes. The lowest anti-hemolytic percentage (27.13%) was reported by *Plumeria obtusa* extract at the lowest concentration compared to other plant extracts or reference standards. Although *Sansevieria cylindrica* resulted in a significant difference when compared with the reference drug, it has reported potent (80.27%) inhibition of hemolysis at concertation of 1.0 mg/ml. The *Plumeria obtusa* extracts at the same concentration were found to be less effective (65.60%) with a highly significant difference of $p< 0.001$ compared to Quercetin. The plants extract in combination demonstrated strong inhibition of hemolysis (93.47% by standard Vs 89.77% by combination) in a statistically non-significant manner. Moreover, the result reported by individual plant extract was similar to that reported using the AAPH reagent, indicating the consistency of results with different assay methods. The results are shown in Table 3. The reported percentage inhibition analysis is displayed in Figure 3.

Table 3: The observations for H_2O_2-induced hemolysis activity (n=3)

Con. (mg/ml)	Sample 1		Sample 2		Sample 1+2		Standard	
	OD	% inhib.	OD	% inhib.	OD	% inhib.	OD	% inhib.
0.2	2.37 ± 0.12	40.44 ± 4.44	2.01 ± 0.08	27.13 ± 2.97	3.04 ± 0.06	65.10 ± 2.06	3.35 ± 0.02	76.57 ± 0.74
0.4	2.88 ± 0.08	59.06 ± 2.80	2.34 ± 0.11	39.33 ± 3.92	3.24 ± 0.04	72.38 ± 1.54	3.52 ± 0.12	82.74 ± 4.32
0.6	3.05 ± 0.07	65.47 ± 2.67	2.37 ± 0.07	40.32 ± 2.67	3.49 ± 0.03	81.87 ± 0.93	3.65 ± 0.05	87.67 ± 1.70
0.8	3.24 ± 0.09	72.38 ± 3.23	2.90 ± 0.04	60.05 ± 1.30	3.53 ± 0.03	83.11 ± 0.93	3.68 ± 0.07	88.78 ± 2.59
1.0	3.45 ± 0.11	80.27 ± 3.91	3.05 ± 0.07	65.60± 2.52	3.71 ± 0.04	89.77 ± 1.30	3.81 ± 0.04	93.46± 1.40

** Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Figure 3: The percentage inhibition of hemolysis induced by H_2O_2



** PE: Plant extract; Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Fibrinolysis

This experiment aimed to assess whether *Sansevieria cylindrica* and *Plumeria obtusa* plant extracts were able to prevent clot formation. Figure 4 shows the in-process fibrinolytic activity using plant extracts.

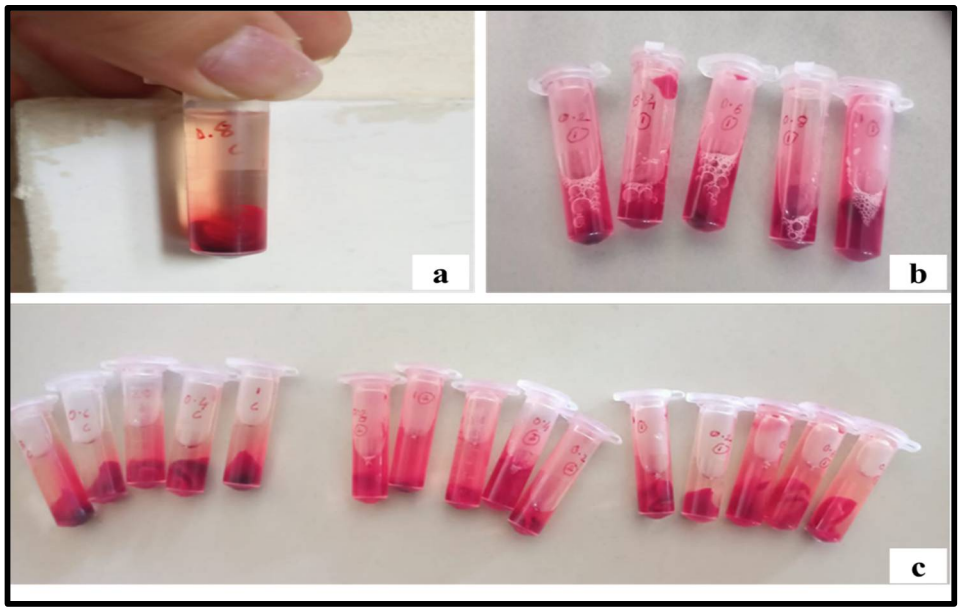
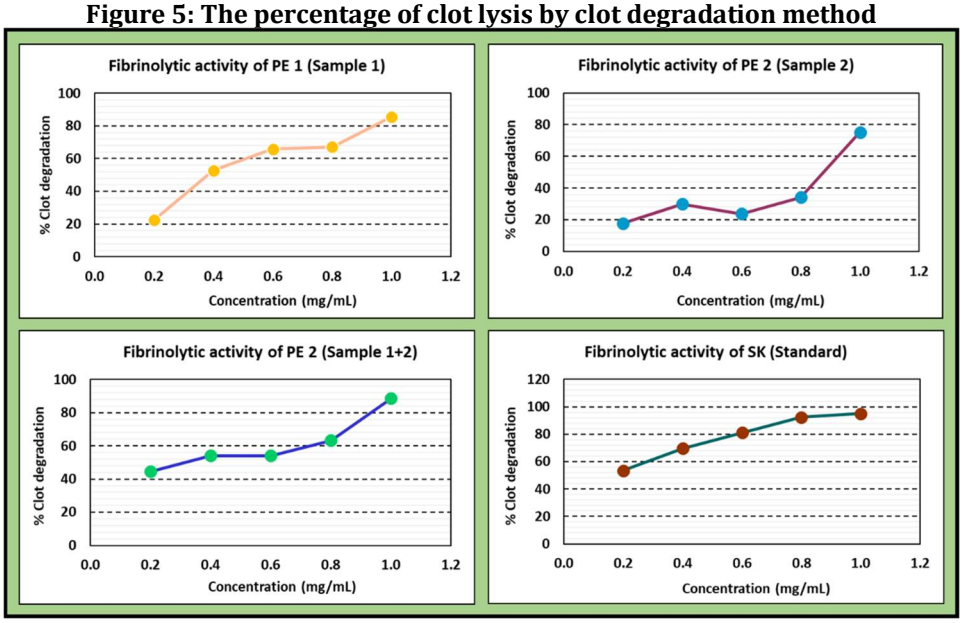


Figure 4: In-process fibrinolytic activity; **a:** Formation of clot after incubation; **b:** Clot tubes containing standard; **c:** Clot tubes containing sample 1, 2 & 1+2.

Fibrinolytic activity by clot degradation

The *in-vitro* fibrinolytic study revealed that at 1.0 mg/ml concentration, extract of *Sansevieria cylindrica, Plumeria obtusa*, their combination, and the standard drug showed 85.62%, 75.20%, 88.59%, and 95.06% clot lysis activity respectively, which were highly significant ($p < 0.001$) compared with negative control (normal saline). The individual plant showed a dose-dependent trend for degradation of a clot in the study. The same pattern was reported when fibrinolytic activity was assessed using reference drugs and plant extracts in combination. The *Sansevieria cylindrica* demonstrated, higher clot lysis but, with a significant p-value of < 0.05 when compared with Streptokinase. In contrast, the *Plumeria obtusa* reported a highly significant difference ($p < 0.001$) in activity with Streptokinase. The synergistic effect was observed in the case of a 1:1 combination of both plant extracts with a non-significant difference from that of the standard. This indicates that both plants reported promising and comparable observations with that of Streptokinase, used as a control. (Table 4) The percentage clot lysis by sample 1, and sample 2, both samples in combination and standard is represented in Figure 5.

Figure 5: The percentage of clot lysis by clot degradation method



** PE: Plant extract; Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Table 4: The observations for fibrinolytic activity by clot degradation method (n=3)

Sample	Concentration (mg/ml)	OD	% fibrinolysis
		(Mean ± SD)	
Sample 1	0.2	0.990 ± 0.003	22.43 ± 0.116
	0.4	1.691 ± 0.080	52.75± 3.449
	0.6	1.992 ± 0.081	65.77± 3.502
	0.8	2.023 ± 0.030	67.09 ± 1.283
	1.0	2.451 ± 0.088	85.62± 3.796
Sample 2	0.2	0.882 ± 0.042	17.77± 1.798
	0.4	1.162 ± 0.174	29.86± 7.521
	0.6	1.023 ± 0.072	23.85± 3.123
	0.8	1.263 ± 0.018	34.26± 0.774
	1.0	2.210 ± 0.053	75.20± 2.271
Sample 1+2 (1:1)	0.2	1.502 ± 0.161	44.57± 6.954
	0.4	1.720 ± 0.025	54.00± 1.060
	0.6	1.721 ± 0.031	54.03± 1.326
	0.8	1.934 ± 0.127	63.24± 5.505
	1.0	2.520 ± 0.025	88.59± 1.060
Streptokinase (Standard)	0.2	1.708 ± 0.211	53.48± 9.135
	0.4	2.083 ± 0.086	69.68± 3.737
	0.6	2.346 ± 0.124	81.08± 5.355
	0.8	2.606 ± 0.041	92.29± 1.781
	1.0	2.670 ± 0.084	95.06± 3.623

** Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Fibrinolytic activity by clot weight

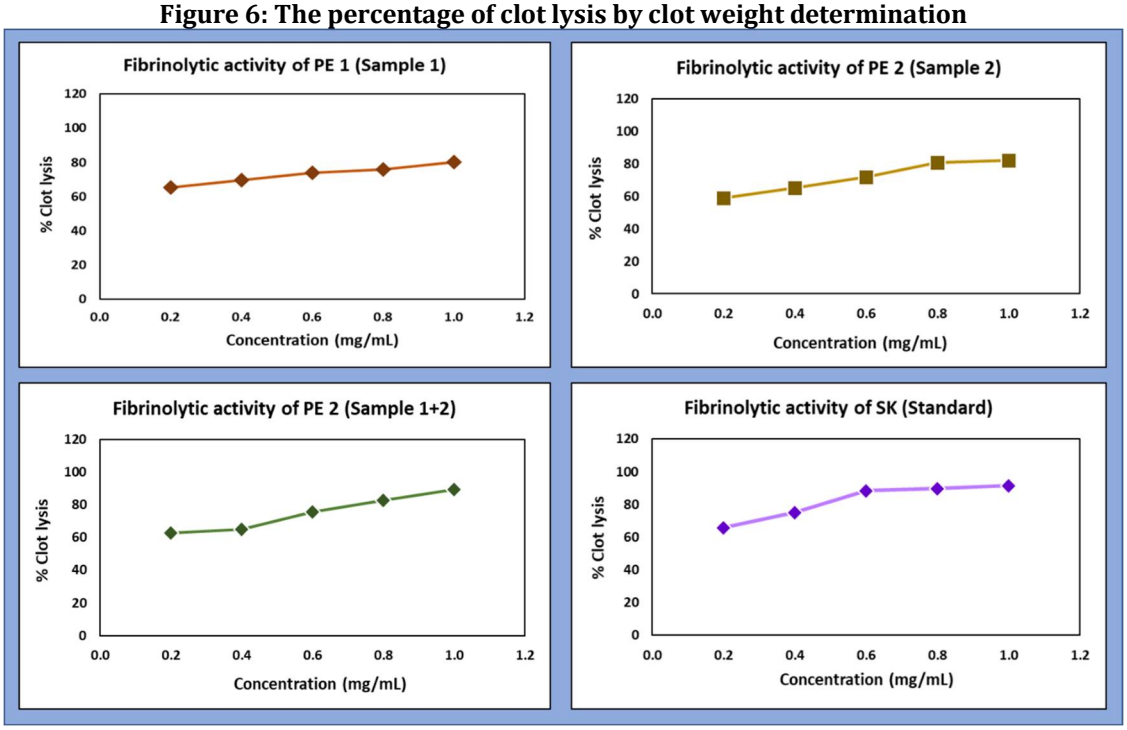
The fibrinolytic activity by clot weight method was higher in *Plumeria obtusa* compared to *Sansevieria cylindrica* plant extract at the concentration of 1.0 mg/ml. The rate of clot lysis was found to be gradually increasing with the increasing concentration of extract. In the case of clot lysis at the lowest concentration used in the study, the Sansevieria was non-significant whereas, Plumeria was reported to be showing significantly less activity than standard. However, the activity of both these plants in comparison with Streptokinase was found comparable. Like, clot degradation assay, here also the combination of plant extract reported higher and statistically significant activity ($p < 0.001$) compared to individual plants (Table 5), indicating an interactive effect between the two. The dose-dependent percentage of clot lysis by plant extracts and reference drugs is exhibited in Figure 6.

Table 5: The observations for clot lysis by clot weight determination method (n=3)

Sample	Concentration (mg/ml)	Initial clot wt. (g)	Final clot wt. (g)	% Clot lysis
Sample 1	0.2	0.1223	0.0798	65.25 ± 0.935
	0.4	0.0875	0.0610	69.75 ± 0.538
	0.6	0.1071	0.0794	74.11 ± 0.289
	0.8	0.1243	0.0943	75.85 ± 0.637
	1.0	0.2031	0.1631	80.28 ± 0.966
Sample 2	0.2	0.1912	0.1127	58.95± 0.659
	0.4	0.1163	0.0758	65.17± 0.869
	0.6	0.1837	0.1320	71.86± 1.417
	0.8	0.1257	0.1015	80.81 ± 0.509
	1.0	0.1321	0.1084	82.05± 0.806
Sample 1+2 (1:1)	0.2	0.1810	0.1132	62.57± 0.884
	0.4	0.1292	0.0839	64.97± 1.060
	0.6	0.1157	0.0873	75.42± 0.869
	0.8	0.1470	0.1216	82.77± 1.059
	1.0	0.1077	0.0960	89.14± 0.471
Streptokinase (Standard)	0.2	0.1690	0.1111	65.76± 1.520
	0.4	0.1134	0.0851	75.02 ± 0.308
	0.6	0.1609	0.1423	88.42± 1.279
	0.8	0.1306	0.1171	89.66± 1.095
	1.0	0.1827	0.1672	91.50± 0.967

** Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

Figure 6: The percentage of clot lysis by clot weight determination



** PE: Plant extract; Sample 1: Sansevieria cylindrica leaves extract; Sample 2: Plumeria obtusa seed extract*

DISCUSSION

Erythrocytes could be the first targets for free radical attack [33]. Hence, the erythrocyte models have been widely used for the determination of toxicity of injectable formulations as well as a general indication of membrane toxicity. Easy availability of blood, simple technique for isolation of cells, and similarities of the membrane with other cell membranes are the added advantages of erythrocyte models [43]. Thus, in this study, an assessment was performed on the ability of plant extracts to protect RBCs from oxidative damage caused by AAPH and H_2O_2-induced hemolysis.

The analysis of linear correlation displayed a significant interaction between hemolytic inhibition activities (AAPH and H_2O_2) and flavonoid content. Based on these results, some of this activity seems to be explained by the presence of polyphenols in plant extracts, especially flavonoids [32]. Our results are also in line with previous investigations that have proven the remarkable antihemolytic activity of several plant extracts, and the capacity of the phenolic content to protect erythrocytes from oxidative stress or to increase their resistance to oxidative-induced damage. Indeed, in a recent scientific work, Bouhlali et al. [42] assessed the protective effect of six Moroccan date varieties from Morocco against AAPH-induced hemolysis and concluded that the *Jihl* variety exhibited considerable antihemolytic activity with a half time of hemolysis (210.99 min) more than the control (175.84 min). Similarly, various plants from Asparagaceae and Apocynaceae families also reported potential anti-hemolytic activity [35, 36]. Moreover, other Sansevieria species were also observed with high membrane stabilization activity [33, 34]. Our findings are also supported by an earlier report wherein nanoparticles synthesized using *Plumeria obtusa* extracts act as RBC stabilizing agents [37].

Different newer techniques including pulsed-laser thrombolysis and synthetic blood clot model are employed for the assessment of *in-vitro* thrombolytic activity [38, 39]. However, these approaches are complex in operation. The concentration of fibrin degraded particles released after clot lysis has been measured by D-dimer assay which is highly accurate, but it is an expensive method [40]. The occluded tube model, which simulates the obstruction of a vessel by a clot, was shown to be unable of discriminating the lower doses of thrombolytic agents while requiring expensive materials [8, 38]. Thus, the current investigation used conventional and common *in-vitro* clot lytic and degradation models to study the effect of plant material as thrombolytic agents. This thrombolytic assay seems to be the preliminary attempt to justify the potential of *Sansevieria cylindrica* and *Plumeria obtusa* plants for clots lysis based on their ethnobotanical information. In this study, the comparison of positive control with negative control demonstrated that clot dissolution does not occur when saline was added to the clot. A comparable thrombolytic profile was obtained when the clot lysis percentage obtained through Streptokinasewas compared with the clots with the plant extracts. The biochemical mechanism of these plant extracts can be explained in the light of the thrombolysis process which may involve the inhibition of

enzymes that are responsible for generating blood clotting proteins or conversion of prothrombin into thrombin [28]. The results are strongly supported by the role of *Sansevieria trifasciata* and *Plumeria alba* L.; (the other species from the Asparagaceae and Apocynaceae family respectively), in thrombolysis [18, 6, 7]. The results are also in accordance with many other reported evidences of plant extracts showing thrombolytic activities [36].

CONCLUSION

In this experiment, there were statistically significant and highly significant differences reported in the case of percentage inhibition of hemolysis by individual plant extracts, but the combination of both the plants demonstrated comparable results without any significant difference with the standard. Similarly, in the case of clot lysis assessment, the combination works better compared to individual plants. Nevertheless, each plant extract also showed potential activity when acted separately.

The present work indicates that plant extracts possess considerable antihemolytic and fibrinolytic capacities. Further studies involving bioassay-guided identification of the main compounds in plants are necessary to affirm and maximize the possible use of the plant for these observed biological activities. These findings justify the traditional uses of both the plants for therapeutic purposes including clinical usage in medicine.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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